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JAMES M. WALTER[2]

AN OUTLINE OF THE PROBLEM

Losses and Possibilities of Control

Common "boil" smut, *Ustilago zeae* (Beckm.) Unger, of corn, *Zea mays* L., is an ever-present disease which regularly exacts a toll on an important crop. In the United States corn is the leading cultivated crop, and conservative estimates place the loss due to smut at two per cent, or approximately 55,000,000 bushels, annually. The disease rarely becomes truly epidemic, but in most corn-growing regions it is present more or less abundantly every year. Recently more attention has been given to the causal organism and the losses induced by it because the disease is assuming greater importance, especially in the drier parts of the corn belt (28).

In small gardens losses owing to smut may, in certain localities, sometimes be appreciably reduced by removing and burning the early galls before they begin to shed spores. However, no practicable method of large-scale control other than the breeding of resistant varieties has been devised, and it is doubtful whether any other method could be practicable, since the disease rarely becomes epidemic and corn is grown on so extensive a scale and under such diverse conditions.

Complications of the Problem of Breeding Resistant Varieties

It is the problem of the corn breeder to synthesize desirable, high-yielding, smut-resistant, commercially reproducible strains of corn from selfed lines. It is only by beginning with the latter that the breeder can work toward this end, because of the heterozygosity of normal corn. Altho progress to date has been relatively slow, selfed lines of corn highly resistant to smut under field conditions have been isolated, and certain sweet corn lines have been combined to produce fairly resistant, high-yielding hybrids of good quality. The mating of apparently resistant field corn lines has failed as yet to produce desirable hybrids

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that are consistently resistant. Kyle (21) has warned corn breeders that the most smut-resistant selfed lines are usually lowest in vigor, and it has been found (14, 15) that F_1's from crosses of high and low smut lines are, in general, intermediate in reaction.

In breeding for resistance to corn smut, the lines and varieties to be tested must be grown under natural conditions for several years to allow recording of their smut responses, because there is no known effective method of artificial inoculation that gives results in close agreement with natural infection. The very effective hypodermic syringe method of inoculation results in production of smut by practically every properly inoculated plant, regardless of its line or variety reaction under natural conditions. The experience with many lines has been that there is considerable variation in their smut responses from season to season (14, 15).

Within recent years the biology of *Ustilago zeae* has received especial attention, and certain of its features have created considerable interest. Stakman and his co-workers (4, 32, 33, 34) have shown that *U. zeae* is heterothallic, that certain lines within it are highly variable, and that, in all probability, many forms differing in pathogenicity exist in nature. They have presented evidence that new forms are constantly being produced by the fungus and have stated that the severity of attack by smut depends upon both the line of corn and the combination of gametic lines of smut involved. Eddins (6) has reported that multisporidial cultures from collections from 12 different localities in Iowa differed in their virulence on several inbred lines of corn.

The Pathology of Corn Smut

Brefeld (2, 3) made signal contributions to our knowledge of the pathology of corn smut 50 years ago. He studied dissemination, infection, and the relationship between the organism and its host in greater detail, perhaps, than anyone up to the present time. He found *(a)* that the organism enters the host by direct penetration, but only through tender, weakly- or non-cuticularized tissues, *(b)* that galls result only from infections in growing tissues, and *(c)* that infection is not systemic, the mycelium being strictly localized in the host. It was Brefeld's conception, as it is ours, that smut galls are produced as a result of parasitic stimulation of host cells to abnormal growth and division, and that cells beyond a certain stage of maturity are not thus stimulated. Brefeld noted that smut galls appear on young leaves, tassels, stalks, nodal buds and adventitious roots of the corn plant. Potter and Melchers (28) consider that the meristem or growing-tissue requirement for the development of galls on all these organs deserves emphasis.

Apparently greatly influenced by his discovery of the rapid, yeast-like budding of the sporidia in nutrient solutions and thinking that chlamydospores would not germinate in water, Brefeld stressed the importance of air conidia as readily disseminable inoculum accountable for all of the infections in nature. Brefeld's emphasis on the function of air conidia as inoculum led to the erroneous conception that aerial conidia falling upon the exposed portions of a plant produce direct local infection, but Potter and Melchers (28) have stated that virulent cultures in the moisture of the leaf axils of young plants are the common sources of infection. They state further that the fungus growing in the leaf axil of a young plant, when the stalk is a cone of meristematic tissue less than two inches tall, would not need to spread very far to account for galls in several places on the fully developed stalk and thus simulate systemic infection. Very recently evidence has been presented (39) that chlamydospores of *Ustilago zeae* are themselves important as inoculum, for they send out germ tubes that cause infection directly; or they may germinate in the leaf-spiral moisture of corn plants to produce sporidial cultures which may be either infectious or innocuous, depending on the biotypes present and contact with an infection court.

The Methods of Approach and the Factors Studied

Since the characteristic galls or "boils" make it one of the most conspicuous diseases, corn smut has been the subject of many sporadic observations but relatively few careful investigations since the time of Brefeld. The literature dealing with the factors influencing the development of corn smut, especially that concerning ecologic relations of the disease, is an array of confusing and contradictory statements. This suggests at once that the appearance and development of the galls are governed by host and environmental interactions which are not as yet well understood. Since a better understanding of the factors involved in smut reaction seemed requisite to a sound and more successful program of breeding for smut resistance, a number of studies, representing different methods of approach and dealing with different phases of the broad problem of nature of resistance, have been made by the writer since the spring of 1930. Data for 1930 (38) are preliminary to those given here.

During the field seasons of 1931, 1932, and 1933 there have been amassed certain data which will, perhaps, support tentative conclusions. In attempting to find a reliable natural method of inoculation, several practices, some involving injury, have been tested as carefully as possible under field conditions, and certain interesting, possibly illuminating, results have been obtained. Observations and data concerning the relation of host vigor to the development of the disease have been

collected from carefully planned field tests involving cultural practices, such as date of planting, methods of cultivation, and application of natural and commercial fertilizers. A number of selfed lines of different degrees of resistance have been studied to see if there could be found any character or index that would materially aid in the selection of resistant lines. Measurements have been taken on these lines to test the idea advanced by Griffiths (9) and Platz (27) that the relative accessibility of the growing tip when the plants are about a foot tall determines the reaction of a plant to smut. Joly balance measurements also have been made on representative plants from a number of the selfed lines to determine the relationship between resistance of their leaves to puncture and their smut reactions.

Since these studies on *(a)* the effects of injury and inoculation, *(b)* the relation of host vigor, and *(c)* the relation of characters of selfed lines to the development of smut are more or less separate and distinct, they will be treated separately in the following pages. Treatment en masse could only lead to greater confusion. The literature pertinent to each unit will be reviewed in sufficient detail to make clear the status of the problem before the experiments in question were undertaken, then a brief discussion of general materials and methods will be given. Following the presentation of the various data and observations under the three inclusive headings, all the results and conclusions will be reviewed in a general discussion to show that interrelation may allow interpretations of greater breadth and lead to a better understanding of certain factors that are important in the development of corn smut.

REVIEW OF PERTINENT LITERATURE

Effects of Inoculation and Injury on Development of Corn Smut

In only one out of many experiments was Brefeld (2) successful in producing smut on a high percentage of plants inoculated by the dropping method, i.e. merely transferring inoculum to the apical leaf spirals. In this case, in which he obtained 100 per cent infection, the plants were started in laboratory boxes in April, transferred to the field in May, and inoculated about the middle of June, when they were only one foot tall. Since he intimates that he used sweet-corn varieties exclusively in this series of experiments, one may assume that the above procedure gave very stocky, loose-spiralled plants in which the apical buds stood relatively high by the middle of June.

Miss Griffiths (9) and Schaffnit and Volk (30) have been unsuccessful in inducing infection by the dropping method of inoculation. Platz (27) was relatively unsuccessful with this method, for only a low

percentage of inoculations resulted in infection, and the reactions of the lines tested did not agree with the field reactions. Eddins (6) compared the dropping method of inoculation with the hypodermic syringe method in the greenhouse and found that only the latter produced galls regularly, altho he reported that 67 per cent of the plants of one line inoculated by the dropping method produced galls. In their field tests both Griffiths (9) and Platz (27) found that the dropping method failed to increase smut above natural infection.

Melhus and Davis (25) reported that addition of about one per cent fish-oil soap to the inoculum to be dropped into the leaf spirals resulted in a high percentage of infected plants and the production of large galls. They considered that reduced surface tension of the liquid in which the sporidia were suspended enabled them to reach the susceptible tissue deep in the rolled leaves.

Immer (15), studying greenhouse plants in 1925, concluded that all lines were susceptible when inoculated by the hypodermic syringe method, regardless of field reaction. Tisdale and Johnston (36) and Immer and Christensen (16) later reported that some lines, resistant in the field, tended to be resistant when inoculated by the syringe method, whereas susceptible lines tended to succumb when thus inoculated. Miss Griffiths (9), however, working in both field and greenhouse, concluded that resistant and susceptible lines and crosses between them were equally susceptible to inoculation by the hypodermic syringe method. The writer (38), in checking syringe inoculations with sporidial suspensions in the field in 1930, found that injection of sterilized water or broth resulted in marked increases in smut.

Platz (27) reported that young ears were readily infected by dropping inoculum into their distal ends, but he found the following practices ineffective: *(a)* dusting plants with chlamydospores, *(b)* spraying them with sporidial suspensions, and *(c)* dropping the sporidial suspensions between leaf-sheath and stalk. The writer (38) also found the latter three practices ineffective in 1930.

Platz (27) concluded that mutilating plants before dusting with chlamydospores or spraying with a sporidial suspension did not induce infection. MacMillan (22) observed that galls which appeared soon after a severe hail-storm in a small area in Colorado were practically all at the nodal buds, even tho the plants were severely torn and bruised. Piemeisel (26), on the other hand, concluded that injury to the host increased the chances for infection, and Clinton (5) found that mutilation by *(a)* detasseling corn just at the time tassels were emerging from the spirals and *(b)* tearing husks away from young ears increased the chances for infection.

The Influence of Ecological Factors and Cultural Practices on the Development of the Disease

1. Soil Moisture and Humidity

Selby and Hickman (31), in 1897, stated that there seemed to be more smut in dry than wet seasons, and Potter and Melchers (28) have reported that smut is more prevalent in dry than in humid seasons and regions. The latter authors doubt that moisture can be a factor in smut development under such climatic conditions as corn requires and state that they frequently found 60 to 80 per cent of the stalks affected in semi-arid regions. Immer and Christensen (16), reporting upon the occurrence of smut in selfed-line nurseries sprayed frequently with spore suspensions, observed that years of heavy smut infection were those of dry-weather conditions as expressed by high percentages of sunshine and low numbers of days of precipitation as great or greater than .01 inches. Platz (27), however, in counting smut on plots of the strain "Iodent" of Reid's yellow Dent corn in 1923 and 1927, seasons with 29 and 28 days of precipitations of .01 or more inches meteoric water, respectively, found 7.9 per cent of the stalks smutted in 1923 and 15.7 per cent smutted in 1927.

Arthur and Stuart (1), altho noting that often less smut occurs in an especially wet than in a dry season, concluded that infection takes place during cloudy days or dewy nights. MacMillan (22) thinks that lack of moisture limits the amount of smut in semi-arid areas. Piemeisel (26) considered that cool damp weather favored infection and that thick planting with consequent shading accounted for the greater prevalence of smut in silo corn than in field corn.

Selby and Hickman (31) recorded the observation that corn on land broken from sod had more smut than corn in a nearby river-bottom field which had been planted to corn every season for nearly 60 years.

Platz (27), inoculating by dropping, and Tisdale and Johnston (36), inoculating by the hypodermic syringe method, both concluded that high relative humidity favored infection in their greenhouse experiments.

2. Manure and Commercial Fertilizers

It has been commonly observed by those (1, 5, 13, 31) following Brefeld (2) that the manuring of corn land favors the development of smut, and it has been recommended by many that manure should not be applied to corn land where damage by smut is feared. The effects of manuring have been considered dual, i.e. manure favors the develop-

ment of smut because *(a)* it harbors and cultivates the pathogen, *Ustilago zeae*, and also because *(b)* it increases the vegetative vigor of the host.

Starr (35), working with sweet corn, has found that commercial fertilizers which favor vegetative vigor of the plant slightly increase smut prevalence, but that phosphates alone reduce the amount of smut.

In greenhouse studies, involving inoculation of course, Schaffnit and Volk (30) found that plants well supplied with nitrogen (N) were much more susceptible than checks, while plants deficient in nitrogen were very resistant. Excesses and deficiences of phosphorus (P) gave similar tho less definite results. Both excesses and deficiences of potassium (K), however, increased the susceptibility of corn to smut. Volk (37), supplementing this study with experiments on the effects of different carbon dioxide (CO_2) concentrations on the reaction between host and parasite, found that increasing CO_2 from 0.03 to 0.5 per cent favored the development of smut on plants given excesses of N, P, and K. The susceptibility of K-deficient and the resistance of N- and P-deficient plants remained unchanged as the CO_2 content of the air was varied. When CO_2 was made an inhibiting factor by raising the concentration to 5.0 per cent, smut development was checked, just as the growth of the plants was checked, but reactions within the fertilizer series remained the same.

3. Cultivation Methods

In 1900 Clinton (5) suggested that unusually frequent stirring of the soil which had grown a smutty crop of corn the previous year accounted for a particular increase of smut in one plot over nearby plots.

Kyle (21), reporting extensive studies on the relation between host vigor and susceptibility to corn smut, stated that "level-planted" corn developed more nodal-bud or potential-ear smut than "furrow-planted" corn and explains that the former was more vigorous. Detailed weights and measurements, as well as notes, were taken by Kyle. He made the very interesting statement that all potential-ear smut occurring on the "furrow-planted" corn was at the second and third nodes, the joints between which the plants were most retarded in growth by furrow planting.

4. Date of Planting

Arthur and Stuart (1) and Piemeisel (26) found more smut on early than on late corn, but Potter and Melchers (28) observed no significant difference in the amount of smut on early and late plantings. Clinton (5) reported less smut on early than on late corn.

The Nature of Resistance of Corn to Smut

The dependence of the smut reactions of selfed lines of corn upon heritable characters has been well demonstrated by the studies of Jones (19), Potter and Melchers (28), Hayes *et al.* (12), Garber and Quisenberry (8), Immer (15), and Hoover (14). It is the judgment of the more recent writers of this list that two sets of factors, the one concerned with morphology, the other concerned with physiology, are accountable for the breeding results noted in their studies. This is despite the fact that no line has yet been proven highly resistant when thoroly inoculated by the hypodermic syringe method, the practice of which would be expected to set at naught the effects of morphology.

It has been pointed out, by Hoover (14), especially, that morphological peculiarities control the smut responses which distinguish certain lines of corn. Miss Griffiths (9) and Platz (27) have independently advanced the idea that the relative accessibility of susceptible parts of the plant to the pathogen is the determining factor in the plant's response to smut, a conception which involves gross morphology. Ranker (29), finding that *Ustilago zeae* made less growth in the extracts from husks, leaves, and stalks of certain resistant lines than in extracts from the same parts of susceptible lines, considered that he had found evidence of smut inhibitors in the resistant lines. Resistance due to inhibiting substances in the juice pressed from the plant parts would, without question, be called "physiological." However, one may soon become deeply involved in the establishment of a line of demarcation between "morphological" and "physiological" characters, and it is not proposed that such a task be undertaken here. It seems sufficient to realize that both terms are relative and that the latter, at least, is applied to a little-known realm.

Kyle (21), summarizing detailed and extensive field-plot studies with corn smut, has reported a general tendency for inverse relationship between host vigor and resistance, while Mazé and Mazé (23) have found a direct relationship in their organic-fertilizer studies with corn carried out for a 30-year period.

GENERAL MATERIALS AND METHODS

Varieties

Two of the most generally useful and yet widely different varieties of Minnesota field corn, Rustler and the Crookston strain of Northwestern Dent, have been used in the major portion of the work herein reported. Rustler is a fairly large, broad-leaved, slow-maturing variety adapted to the southern part of the state. The Crookston strain of

Northwestern Dent is a small, early-maturing strain of tillering habit, adapted to the most northern corn-growing sections of the state. When grown side by side in most of the plots used in studies of the effects of inoculation, injury, methods of cultivation, and date of planting on the development of smut, these two varieties responded differently in many cases and permitted observations that are highly important to the interpretation of the results.

Culture and Preparation of Inoculum

Most of the data to be presented are for natural inoculation or infection. The sporidial suspension used in inoculations was a mixture of four monosporidial lines known to produce smut galls in four combinations between themselves. These lines were increased separately in 0.5 per cent Troemmer's malt extract broth (0.5 per cent malt extract in tap water), and, to make the suspension used as inoculum, the broth cultures were strained through a double layer of cheese-cloth and diluted with from four to eight parts tap water, depending upon the age of the broth cultures. Except where otherwise stated, the chlamydospores used as inoculum were spores of the previous year's crop, collected at the end of the season and stored in a dry place during winter.

The Method of Representing Severity of Smut

As the studies progressed, it became more and more evident that the commonly employed method of representing smut reaction, i.e. by the percentage of stalks smutted, a method which gives prevalence but not severity, was not at all adequate. It appeared that the figure to be desired for representing the amount of smut on a given plot must include both size and number of galls and thus be a product value.

On certain material developed in their extensive studies of the reaction of lines and varieties of corn to *Ustilago zeae,* Immer and Christensen (17, 18) have used refined technic to arrive at accurate estimates of the reductions in the yields induced by galls of different sizes. They estimate that a gall four inches or more in diameter (large) reduces the yield of shelled grain 50 per cent, a gall two to four inches (medium) 25 per cent, and one less than two inches (small) 10 per cent. If the note-taker records galls by these sizes and counts the number of stalks on the plot, a relative product value which gives the severity of smut per stalk and the reduction in yield can be readily calculated as follows: Multiply the number of small galls by 1, the number of medium-sized galls by 2½, and the number of large galls by 5. Divide the sum of these products by the total number of stalks in the unit under consideration, and the quotient will be a relative figure

which gives the average size of the gall per stalk, i.e. 1.0000 would mean an average of one small gall per stalk. Recalling that Immer and Christensen estimated that a small gall causes 10 per cent reduction in the yield, it is readily seen that this relative product value represents the percentage loss or reduction in yield if the decimal point is moved one place to the right to correct for dropping the units place in order to facilitate multiplication.

If one wishes a record of the prevalence of smut, the percentage of stalks smutted, in addition to the severity, he may easily take the necessary data in conjunction with the gall classifying system outlined above by keeping a record of the number of galls above one per smutted stalk; then by subtracting the total of these extra galls (small, medium, and large) from the total number of galls, he obtains the number of smutted stalks. With the exception that prevalence has been calculated in a few cases only, all data taken in 1932 and 1933 have been handled in this manner, an example of which follows:

June 15 planting, series III, Rustler:

Number of stalks	Smutted stalks	Galls			
		Large	Medium	Small	Extra
394	150	21	80	97	48
Multiplying by		5	2½	1	
Summing the products		105	200	97	
		200			
		97			
Total product value		402			

402 ÷ 394 = 1.0203 Move decimal one place to right

150 ÷ 394 = 38.07%

Summary

Severity	Prevalence
10.203	38.07%

The number of smutted stalks equals the total number of galls (21+80+97) less the number of extras (galls above one per smutted stalk =48) =150.

Field-plot Technic and Note-taking

Either randomized-block or Latin-Square arrangement of plots was employed wherever it seemed at all practicable, and in these cases the data were subjected to Fisher's methods (7, 10), "analysis of variance."

Plot size is a problem which is complicated by the fact that in some seasons the natural distribution of smut presents an error which is akin to the error introduced by soil heterogeneity (31). It will be noted that plot size was increased each season as the work was carried further. The writer does not consider plots of 400 to 600 stalks of normal variety corn too large for such studies.

Data taken in 1931 represent the percentage of stalks smutted, or prevalence; but the data for 1932 and 1933 are in terms of the calculated severity of smut, the percentage reduction in yield. During the latter

two seasons the records were taken and summarized as in the example given, except in cases for which detailed information on the location of the galls was desired. In such cases the same system was used with the particular portion of the plant as a sub-unit.

EXPERIMENTAL SECTION I

Effects of Inoculation and Injury on Development of Corn Smut

From the brief review of literature concerning the smut reactions of inoculated and injured corn the following facts are apparent: *(a)* The highly artificial hypodermic syringe method is the only method of inoculation that has given consistent results; *(b)* immunity to corn smut is lacking or extremely rare; and *(c)* there is no agreement concerning the relation of injury to the development of smut. With the elucidation of the latter and other problems of infection by *Ustilago zeae* and the development of a natural and effective method of inoculation as the two principal objectives, extensive field inoculations were made in 1931, 1932, and 1933. A dozen studies bearing upon these problems are delineated in the following pages.

1. Inoculation and Injury Tests Made in 1931

In 1931, a very dry season, Rustler and Northwestern Dent were planted on four dates, May 12, May 30, June 20, and July 6. At two leaf-spiral stages, heights 10 to 15 inches and 18 to 24 inches, quadruplicate (excepting non-replicated June 20 corn) plots of the four plantings were treated as follows:

1. Sterilized soil dropped into leaf spirals
2. Sterilized soil plus sporidial suspension dropped into spirals
3. Sporidial suspension dropped into spirals
4. Sporidial suspension plus one per cent fish-oil soap dropped into spirals
5. Sterilized broth plus one per cent fish-oil soap dropped into spirals
6. Sterilized water injected by the hypodermic syringe method
7. Growing points injured with the needle of hypodermic syringe
8. Injured by slashing with a brush of nails
9. Injured by slashing with a brush of nails and sprayed with sterilized water
10. Injured by slashing with a brush of nails and sprayed with chlamydospores

Northwestern Dent (Crookston strain) tassels soon after the 18- to 24-inch stage, but Rustler tassels from one to two weeks later. When Rustler was fairly well tasseled, plots of a third series of each of the four plantings were subjected to treatments Nos. 8, 9, and 10 of the above list, and, in addition, sporidial suspension was dropped on young silks (treatment 11) and injected into young shoots (treatment 12).

The results of this test, involving 325 plots, may be summarized very briefly, perhaps as well without presentation of the bulky and highly variable data. The following seemingly worthwhile conclusions may be listed: *(a)* Treatment No. 6, the injection of sterilized water, was the only practice which gave clear-cut increases in both prevalence and severity of smut, and its effectiveness increased with the advance of the season; *(b)* as in 1930 (38), certain plots given treatments Nos. 8, 9, and 10 at the 18- to 24-inch stage were quite distinctly more heavily smutted than checks, but the differences were not consistent; *(c)* the statement of Melhus and Davis (19) regarding the function of fish-oil soap was not corroborated; there was no evidence whatever that the fish-oil soap increased the prevalence or severity of smut.

In these tests made in the exceptionally dry season of 1931, each plot contained only 15 to 30 stalks of each of the two varieties, and variability was great; otherwise smaller differences might have been observable and statistically supportable.

2. Randomized Block Test of Injury and Inoculation Practices on Early Planting in 1932

With the generally negative results of the 1931 inoculation and injury tests in the background, it was decided at the beginning of the 1932 season that larger plots, containing from 40 to 50 stalks of each variety, would be used and that inoculation and injury would be done on rainy days, preferably during rain. In greenhouse tests made during the winter, sporidial suspension plus fish-oil soap had failed to cause more smut galls than sporidial suspension alone. It was decided to omit this and other highly artificial treatments from the 1932 trials and, in general, to give closer attention to the more natural methods. Nevertheless, the surprising results from the injection of sterilized liquids during 1930 and 1931 seemed to warrant a more detailed study of the effectiveness of this procedure, which was in the beginning a check on the hypodermic syringe method of inoculation.

Corn of the two varieties previously described was planted May 17. June 30 was the first rainy day after the plants had reached the 10- to 12-inch stage, and by that time the tassels of Northwestern Dent were beginning to show. They were, however, just about the desired stage for No. 6 of the following list of treatments practiced on this corn:

1. Sterilized water injected by the hypodermic syringe method
2. Injured by slashing with a brush of nails and sprayed immediately with chlamydospore suspension
3. Injured by slashing with a brush of nails
4. Check—no treatment
5. Young silks sprayed with sporidial suspension
6. Mutilated by topping, or pulling out emerging tassels

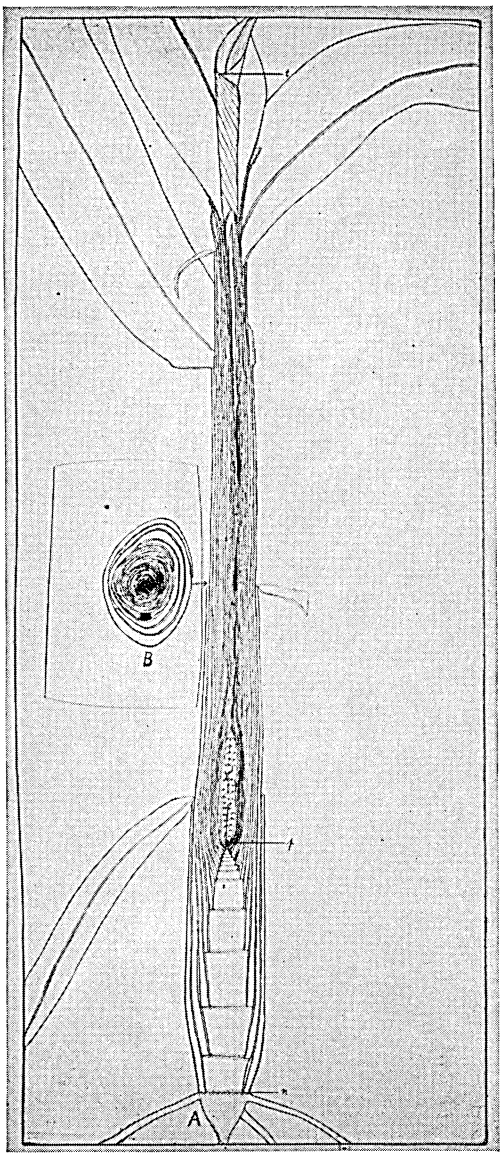

Fig. 1. A. Scale diagram (approx. 0.5 ×) of the corn plant about one foot tall in median longitudinal section: *n* is considered the first node, *p* is the last or youngest node, and *t* is the level at which the spiral could hold water. For the calculation of the growing-point index *n* to *t* was taken as plant height and *n* to *p* as growing-point height.

B. Diagram of transverse section of the corn plant at the point on A indicated by the arrow. (Approx. 0.8 ×)

Treatments 2 and 3 were administered to both varieties and treatment 6 to Northwestern Dent during the rain on June 30. It had ceased raining before No. 1 could be done, and, of course, No. 5 could not be practiced on either variety until later in the season. The emerging tassels were pulled from Rustler just before a rain on July 9, and cloudy weather with very light showers came on August 2 at about the best time for the inoculation of a majority of the young ears of both varieties in the silking stage. Earlier and later visits were necessary to find some of the shoots in the early silking stage, and at these times the inoculum was applied without concurrence of rain.

The results of this series of inoculations are presented in Tables 1 and 2, the figures representing the severity of smut or the percentage reduction in yield calculated by the product method previously described. The plots of different treatments were randomized in each series or block, and the data have been analyzed by Fisher's "analysis of variance" method (7). For the data presented in Table 1 the

analysis indicates that the chances are greater than 19 to 1 that there are differences due to treatments, since the value of Z exceeds the 5 per cent point. In other words, a difference of 4.274 between means could be expected only once in 20 or more trials on the basis of chance. Thus it may be considered that treatments 2 and 3, involving injury, have induced increased severity of smut on Rustler. For the data presented in Table 2, the value of Z greatly exceeds the one per cent point. Unquestionably, therefore, differences of 4.906 between means may be accepted as significant, for the analysis indicates that chances are greater than 99 to 1 that treatments have induced differences. It is evident that treatments 2, 3, and 6 have brought about increases in smut severity above the check in Northwestern Dent.

It is interesting to note that the spraying of chlamydospores on injured plants has not increased the severity of smut above injury alone and that pulling emerging tassels was distinctly effective in producing smut on Northwestern Dent but not on Rustler. The Rustler is, however, nearer its full stalk size when its tassels are loosened by the protecting leaves than is Northwestern Dent.

It is of great interest and importance to note that the increased amount of smut on the mutilated corn was not on the injured surfaces but in the lateral and inter-nodal meristems. This was true of corn mutilated both by detasseling and by slashing with the brush of nails. For the former, the increases were predominantly in neck smut, which develops from intercalary meristems of the nodes between ear and tassel. For the latter, the increases were largely represented by nodal-bud and ear smut, and the increases were most striking on stalks which were so severely injured that the main stalk developed very little further. At the time of injury, the corn was growing vigorously, and considerable elongation was yet to take place.

Table 1.—The Severity of Smut on Rustler Corn, Planted May 17, 1932, and Injured or Inoculated in Various Ways

Treatments*	Series and severity of smut†				Mean‡
	1	2	3	4	
1. Sterilized H_2O injected	5.000	5.122	4.457	2.206	4.196
2. Injured and sprayed with chlamydospores	15.417	10.306	5.893	6.979	9.649
3. Injured	12.789	8.462	7.273	11.705	10.057
4. Check—no treatment	4.518	2.273	2.765	5.060	3.654
5. Young silks sprayed with sporidia	7.500	4.302	6.324	8.488	6.654
6. Emerging tassels pulled	5.405	12.750	5.581	.938	6.169

* For details of treatments see pp. 16 and 17.

† For explanation see p. 13.

‡ A mean difference of 4.274 is required for significance.

Table 2.—The Severity of Smut on Northwestern Dent Corn, Planted May 17, 1932, and Injured or Inoculated in Various Ways

Treatments*	Series and severity of smut†				Mean‡
	1	2	3	4	
1. Sterilized H_2O injected	11.630	10.784	12.317	10.758	11.122
2. Injured and sprayed with chlamydospores	20.244	15.000	13.529	17.708	16.620
3. Injured	13.125	17.500	20.957	19.186	17.692
4. Check—no treatment	5.688	10.460	9.056	8.295	8.375
5. Young silks sprayed with sporidia	9.194	9.674	6.410	11.591	9.217
6. Emerging tassels pulled	23.649	23.026	24.130	13.333	21.034

* For details of treatments see pp. 16 and 17.
† For explanation see p. 13.
‡ A mean difference of 4.906 is required for significance.

3. Injury and Inoculation of Later 1932 Plantings at Different Stages of Growth

On the May 30, June 15, and June 30 plantings of 1932, the following inoculation and injury practices were tried:

1. Injured by slashing with a brush of nails
2. Injured by slashing with a brush of nails and sprayed with chlamydospores
3. Dry chlamydospores dusted over spiral heads
4. Sterilized water injected by the hypodermic syringe method
5. Sporidial suspension dropped into spirals
6. Sporidial suspension plus soil dropped into spirals
7. Soil dropped into spirals
8. Emerging tassels removed by pulling

Only one series of the May 30 planting was treated, and it happened that all of this could not be done during one rain, when the plants were of the same age. Nos. 1, 2, and 4 were done on June 30 when the plants of both varieties of the May 30 planting were 8 to 10 inches tall; and Nos. 5, 6, 7, and 8 were done just before a wind and rain storm on July 9, when many of the Northwestern Dent plants were tasseling. On the latter, the soil and sporidial suspension which was to have been dropped into spirals was dropped between sheaths and stalks. Treatment 3 was not made on the corn planted May 30 because it was not decided to test dusting with chlamydospores until it appeared from observations in the selfed-line nursery that this practice had possibly caused infection of a fair percentage of the plants of certain lines. The results on this one series of May 30[3] corn are included in Table 3 with the results of inoculations of June 15 and June 30 plantings because they furnish some interesting and perhaps significant comparisons with results on earlier and later corn. An example of this is the effect of detasseling the May 30 Rustler at the same time, July 9, as Rustler planted May 17 was detasseled. (See Table 1, treatment 6.)

[3] Plantings are referred to by date in many cases to avoid repetition of word "planting."

Table 3.—The Severity of Smut on Rustler and Northwestern Dent Corn Planted at Three Dates in 1932 and Inoculated and Injured in Different Ways

Treatment	May 30*		June 15†		June 30†	
	Rustler	N. W. Dent	Rustler	N. W. Dent	Rustler	N. W. Dent
1. Injured by slashing with a brush of nails	9.902	7.983	15.757 ± .758	17.078 ± .905	9.101 ± 1.177	11.446 ± .679
2. Injured by slashing with a brush of nails and sprayed with chlamydospores	5.784	6.442	12.673 ± .581	16.197 ± .647	8.775 ± .730	15.699 ± 1.073
3. Dry chlamydospores dusted over spiral heads	.....	.....	5.267 ± .404	9.725 ± 1.101	8.420 ± .928	9.679 ± .497
4. Sterilized water injected by the hypodermic syringe method	3.587	14.697	17.943 ± 1.059	18.051 ± 1.536	26.817 ± 2.113	30.447 ± 1.846
5. Sporidial suspension dropped into spirals	2.881	2.447	8.586 ± .892	11.213 ± .638	6.819 ± 1.297	11.100 ± 1.618
6. Sporidial suspension plus soil dropped into spirals	2.182	5.000	5.835 ± .660	11.113 ± 1.775	7.933 ± .928	8.183 ± 1.648
7. Soil dropped into spirals	3.646	4.681	9.537 ± .680	8.917 ± .892	9.331 ± .762	12.473 ± 1.166
8. Emerging tassels removed by pulling	13.056	8.095	9.637 ± .798‡	21.116 ± 1.221	7.256§	6.633‖
Check	4.937	8.108	10.291 ± .793	12.464 ± .556	8.486 ± .609	9.637 ± 1.071

* Only one series. Treatments 1, 2, and 4 practiced June 30 on plants 8 to 10 inches tall; Nos. 5, 6, 7, and 8 practiced July 9 with Northwestern Dent tasseling, thus for this variety the soil and sporidial suspension was dropped between leaf sheaths and stalks.

† Both plantings treated July 25, the June 15 plants being 20 to 24 inches tall and June 30 plants 10 to 12 inches tall.

‡ Tassels pulled August 2.

§ Only one row; detasseled August 24.

‖ Two rows only; fully extended tassels clipped August 27.

Corn of the June 15 planting was 20 to 24 inches tall and that of the June 30 planting was 10 to 12 inches tall on July 25. The weather on July 25 was perhaps as nearly perfect as possible for the purpose of these studies. A gentle rain started about daylight, continued until 11:00 a.m., and, with the exception of about 30 minutes of weak sunshine between 2:00 and 3:00 p.m., the remainder of the day was dark and damp. Treatment No. 4 was administered during the afternoon, and at about 10:00 p.m., after a heavy dew had formed, tassels were pulled from series 2, 3, and 4 of the June 15 Northwestern Dent. The remaining inoculation and injury treatments, excepting, of course, removal of tassels from the May 30 planting and June 15 Rustler, were completed before the showers ceased. Thus the results of these treatments on the June 15 and June 30 plantings, presented in Table 3, offer good comparisons for the determination of the influence of stage of development of corn on its responses to injury and inoculation, and, at the same time, show the effects of each method on each variety at a certain stage of development.

The data presented in Table 3 warrant, in the writer's opinion, a number of observations, some of the most striking of which are:

1. Pulling the tassels from the May 30 Rustler markedly increased the severity of smut, but pulling tassels from Northwestern Dent of the same planting and age was ineffective. It is noteworthy that the tassels of this Rustler were still protected or ensheathed by one or two rolled leaves when they were removed, but the tassels of the earlier maturing Northwestern Dent were too far out to be properly termed "emerging."

2. Injection of sterilized water was ineffective on Rustler of May 30 but increased the amount of smut on Northwestern Dent. This treatment was applied when the plants were only 8 to 10 inches tall, and at this stage the cone of growing tissue in Rustler is lower than in Northwestern Dent. (See Fig. 1.)

3. Injection of sterilized water and injury by slashing, the latter with and without the spraying of chlamydospores, increased the severity of smut on the June 15 Rustler and Northwestern Dent (Fig. 2).

4. Pulling the tassels from the June 15 Northwestern Dent (Fig. 2) led to a marked increase in smut, but pulling the tassels from Rustler of the same planting and Rustler of the June 30 planting had no apparent effect on smut development.

5. On the June 30 Rustler, injection of sterilized water was the only practice that increased the severity of smut; the increase was marked, as was true also for Northwestern Dent of June 30.

6. Injuring the June 30 Northwestern Dent, followed by spraying with chlamydospores, increased the severity of smut, but injury alone

failed to cause significant increase. A small increase over the check due to treatment 1 was apparent, but it is not sufficient that the difference is three times its probable error. The difference between treatments 1 and 3 on this planting is, however, significant, i.e. by chance alone it could be expected only once in 20 or more trials; nevertheless, it is the only case in which the spraying of the injured plants with chlamydospores has significantly increased the smut above injury alone and, until further tests are made, can only be considered chance.

Fig. 2. The Effects of Mutilation on Smut Development in Normal Corn
A. Northwestern Dent planted June 15 and topped or detasseled July 25, 1932. Four plants each of checks and mutilated; checks left.
B. Rustler planted June 15 and slashed with a brush of nails on July 25, 1932. Two plants each of checks and mutilated; checks left.

7. There was no indication whatever of increased severity of smut on plants of Northwestern Dent from which tassels were clipped.

8. Dropping soil, chlamydospores, and sporidial suspension into leaf-spirals not only failed to increase smut severity but actually resulted in significant decreases in the following cases: For the June 15 planting of Rustler the means of treatments 3 and 6, dry chlamydospores and soil plus sporidial suspension dropped, respectively, are significantly less than the check. The same is true for Northwestern Dent under treatment 7, soil alone dropped. It seems that treatments 5, 6, and 7 have all reduced smut on the May 30 planting.

9. Injury of the May 30 Rustler and Northwestern Dent when the plants were 8 to 10 inches tall and the June 30 Rustler when the plants were 10 to 12 inches tall did not increase smut (despite the difference between May 30 Rustler check and treatment 1), but the injury of both

varieties at the 20- to 24-inch stage (June 15 and May 17 plantings) resulted in pronounced increases. Injury of June 30 Northwestern Dent at the 10- to 12-inch stage increased the severity of smut on it, however. These results are considered good evidence that stage of development of the host plant is of vital importance to the increase of smut due to injury.

10. The injection of sterilized water was ineffective on the May 30 Rustler but effective on Northwestern Dent when both varieties were 8 to 10 inches tall.

11. The injection of sterilized water was distinctly more effective in producing smut on June 30 corn (10 to 12 inches tall) than on June 15 corn (20 to 24 inches tall) treated the same day. The location of the resultant smut was different on the two plantings of Rustler (see Table 8), and it seems clear that stage of development is very important in determining the differential responses to this treatment.

12. Recalling the insignificant increases of smut from injection of sterilized water into the May 17 corn (see Tables 1 and 2) for comparison with the results of this practice on the June 15 corn, treated at the same stage (20 to 24 inches), it seems that the data support the 1931 observation that the effectiveness of injection of sterilized water increases with the advance of the season.

Pulling the tassels from the May 30 Rustler on July 9 without doubt brought about an increase in the amount of neck smut on this variety, regardless of the size of plot (54 stalks) and the lack of replications. There was more smut on Rustler in this plot than the writer has seen on this variety except when inoculated by the syringe method. This exceptional case of smut increase on Rustler due to detasseling perhaps deserves detailed consideration. Removal of tassels, by the same method and on the same date (July 9), from Northwestern Dent of the same planting and Rustler of the May 17 planting failed to bring about increases in smut. It must be made clear, moreover, that the May 30 planting had developed much more rapidly than the May 17 planting and was not, on July 9, thirteen days behind the earlier corn in stage of development. It has been stated that tassels were emerging from May 17 Northwestern Dent plants on June 30 but that the May 30 Northwestern Dent was tasseling on July 9. This difference in tasseling stages means a difference of about two days. Thus we can assume that the May 30 planting was seven or eight days short of the stage of development attained by the May 17 planting. When coupled with other observations, this slight difference in stage of development of the two plantings of Rustler similarly treated seems quite important to the result and its interpretation. The only justifiable conclusion is that neck

smut develops on Rustler plants if they are topped early enough in their development.

4. Injury and Inoculation in 1933

Even tho the results of injury and inoculations in 1932 seemed fairly conclusive, it was deemed best to try several of the methods again in 1933 and to add a treatment which simulates the spiral-loosening effect of the severe twisting in the wind, namely, rolling the young stalks between the palms of the hands. Using plots in randomized block order, each containing approximately 40 stalks, Rustler and Northwestern Dent planted on May 17, June 6, and June 24 were treated as follows:

1. Stalks rolled between hands during or immediately following rain
2. Sterilized water injected following rain
3. Detasseled during or immediately following rain
4. Topped during or immediately following rain
5. Dusted with chlamydospores at approximately four-day intervals from the 8- to 10-inch stage to tasseling
6. Injured by slashing with a brush of nails during rain
7. Check—no treatment

The results of these practices on the three plantings of two varieties are summarized in Table 4. The following results are noteworthy:

1. Rolling of the stalks failed to produce increases of smut on the early planting but was distinctly effective on both varieties of the late planting and caused significant increase on the Northwestern Dent of the second planting.

2. Injection of sterilized water was much more effective in increasing smut on June 6 corn than on either the earlier or later plantings. This treatment resulted in significant increases on Northwestern Dent but not on Rustler planted May 17 and June 24. Can the slower unfolding of Rustler, the greater inaccessibility of the tassel and other tender tissues of young plants less than 12 to 15 inches tall be the reason for this difference?

3. The pulling of tops (tassels protected by rolled leaves) was distinctly more effective than the removal of emerged tassels in leading to increased smut on the May 17 planting, the difference between the means for the two treatments being statistically significant for Rustler only.

4. Injury by slashing was, on all plantings, more effective on Northwestern Dent than on Rustler, i.e., greater smut increases are noted for Northwestern Dent.

5. In five out of six cases the means of plots dusted with chlamydospores are lower than those of checks, but, of course, are not significantly less if we assume that only the differences set by the biometrical analysis are worthy of consideration. The writer believes that there must be a

reason for the frequently noted lower severity of smut on heavily dusted plants but can only speculate upon it at present.

Within 10 to 15 days after treatment, it was evident that rolling the young stalks (leaf-spiral stage) between the hands had induced increases in leaf and tassel smut in every plot thus treated, despite the fact that significant increases are not shown for Rustler of May 17 and June 6 and Northwestern Dent of May 17. On the plants of these three series of plots the galls were small, and, by the time the corn was mature, many were no longer visible because small tassel galls often drop off, and the drying and dropping of leaves results in the disappearance of small galls on them.

Careful review of the results presented in Tables 1, 2, 3, 4, and 8, with their supporting observations, leads one to two general conclusions regarding the influence of inoculation and mutilation of the host on the severity of smut, viz.: *(a)* Mutilation markedly increased the smut severity when the corn was mutilated at an intermediate stage of development, the limits of the period of effectiveness apparently being quite narrow with respect to the six or eight weeks required for the plants to reach full stalk development. The important increases occurred at lateral or internodal meristems. *(b)* Spraying, dropping, pouring, or dusting sporidia or chlamydospores (or both) on the exposed portions of the plant, including those portions reached by meteoric water, did not increase the prevalence and severity of smut above that appearing under natural conditions.

These conclusions are based on results with only two varieties, during only four seasons, and they approach the unbelievable in certain respects. It is therefore desirable to examine every available fragment of evidence which might lead to a better understanding of the phenomena involved, especially *(a)* the influence of the stage of the host and *(b)* the time of the infections which account for galls on the various parts of the plant. The results of several experiments made to obtain further information on these questions are given below.

5. Selfed-line Detasseling

The results given above were, of course, not available until fairly late in the 1932 growing season; however, a series of selfed lines of known reactions, planted July 6, was then available for further study. It is well known that selfed lines of *Zea mays* generally lack vigor, and this was true of those now under consideration. The number of plants per line (10 to 40) was unfortunately so low that it seemed feasible to leave check plants in only 8 of the 77 lines, for 10 to 20 stalks, even with practically homozygous lines, would seem to be the lower limit of

Table 4.—The Mean Severities of Smut on Three Plantings of Rustler and Northwestern Dent Corn Inoculated and Injured in Different Ways in 1933

Treatment	Date of planting											
	May 17*				June 6†				June 24‡			
	Rustler		N. W. Dent		Rustler		N. W. Dent		Rustler		N. W. Dent	
	Date of treatment	Smut severity	Date of treatment	Smut severity	Date of treatment	Smut severity	Date of treatment	Smut severity	Date of treatment	Smut severity	Date of treatment	Smut severity
1. Stalks rolled between hands	June 22, 27	5.25	June 22	6.11	June 27, July 1	8.73	June 27, July 1	18.22	July 19, 23, 31	8.50	July 19, 23, 31	9.30
2. Sterilized H_2O injected	June 21	5.00	June 21	10.72	July 1	18.54	July 1	29.44	July 11	5.26	July 11	8.34
3. Detasseled	July 8	4.87	June 30	7.75	.....	.....	July 10	14.33	August 4	6.06	July 31	7.66§
4. Topped	June 30	9.72	June 20	9.87§	July 10	11.47§	.....	.....	July 31	6.04	.....	.....
5. Dusted with chlamydospores	June 21, 27, 30	2.45	June 21, 27, 30	3.52	June 27,30, July 11	5.00	June 27,30, July 11	11.58	July 11, 24, 31, August 4	3.16	July 11, 24, 31, August 4	4.87
6. Injured by slashing	June 20	6.50	June 20	14.53	June 30	10.85	June 30	17.50	July 19	6.05	July 19	8.96
7. Check—no treatment		4.48		5.72		7.46		10.03		3.76		5.18
Difference between means of column above which may be considered significant‖		3.80		4.49		4.74		7.48		1.71		2.86

* Plants were 15 to 18 inches tall on June 20. Four series.

† Plants were 10 inches tall on June 27, 15 inches tall on June 30. Northwestern Dent was tasseled on July 10, while Rustler was only in the boot on that date. Four series.

‡ Plants were 8 to 10 inches tall on July 11, 20 to 30 inches tall on July 23. Three series.

§ Means that are sufficiently greater than the check to be considered indicative of increases even tho short of the level set by the analysis.

‖ Determined by Fisher's "analysis of variance" method (7).

sample size in studying such a disease as corn smut. In order to make sure that every plant of this series was supplied with abundant inoculum, the plants had been heavily dusted with chlamydospores at four-day intervals from the time they were 8 or 10 inches tall until they tasseled. Spores collected from the early galls on nearby corn were used. On August 24, a day which promised rain, most of the lines were beginning to tassel. Before the rain started the stage of tasseling of each line was noted (see Table 5), and, as the rain began, tassels or tops were removed from all.

The weather was not at all favorable for the rapid growth of corn during the next two weeks, and the resultant galls were small. Since checks were not available in every line, it seemed best, for the purposes of analysis and as a basis for comparisons, to take an arbitrary level of neck-smut severity, 3.000, a level above which it was obvious that detasseling or topping had increased the severity of neck smut. This procedure is based on the assumption that the greatest response to detasseling is in the meristems above the ear, which may not be true for some lines.

Fig. 3. The effects of detasseling on smut development in certain selfed lines. Three plants each of checks and mutilated; checks left.
A. S-126, a Northwestern Dent line selfed 7 years.
B. S-116, a Rustler line selfed 8 years.

It will be noted from the data of Table 5 that 13 of the 77 lines had neck-smut severities greater than 3.000. Classification of the 77 lines by the stage of tasseling recorded for August 24 shows that within the 13 neck-smut lines there are represented: 6 out of 38 that were in

the tasseling stage (neck elongating and tassel rising rapidly), 6 out of 14 that were showing tassels (leaves unrolled sufficiently so that low set tassel was visible), and 1 out of 5 that were not tasseling when the plants were mutilated. Twenty lines which were classified as tasseled on August 24 did not give a single neck-smut line, and 40 of the 77 detasseled or topped lines produced no neck-smut. Evidently, then, the effect of this type of mutilation varies with the line of corn (Fig. 3), and again the results strongly suggest that the stage of the plant at the time of mutilation is an important factor.

Table 5.—Stage of Tasseling, Smut Severity, and Ear Bagging Results on Selfed Lines of Known Reactions Detassled August 24, 1932

Line number (1930)	3- to 5-year average reaction		Stage when topped or detasseled August 24	Smut severity at end of 1932 growing season		Number ears bagged September 1	Bagged ears smutted end of growing season
	Total	Ear		Neck	Total		
1	10.2	.....	Showing tassels	0.476	1.667	.....	.....
5	52.1	14.9	Tasseled	0.345	3.276	10	0
6	9.5	.....	Showing tassels	5.000	7.917	.....	.....
7	8.7	.....	Showing tassels	6.000	11.000	.....	.....
18	27.4	.....	Tasseling	0.000	5.000	.....	.....
19	29.0	.....	Tasseled	0.000	1.250	.....	.....
21	24.9	.....	Not tasseling	0.500	15.750	.....	.....
22	22.3	.....	Tasseled	0.000	0.000	.....	.....
25	8.0	.....	Tasseled	0.000	0.000	.....	.....
32	2.8	.....	Tasseling	2.000	4.000	.....	.....
32	Check	.....		0.000	4.231	.....	.....
33	9.5	.....	Tasseled	1.091	6.957	.....	.....
34	23.2	.....	Showing tassels	3.333	13.000	.....	.....
34	Check	.....		0.000	7.917	.....	.....
40	3.0	.....	Tasseled	0.000	2.813	.....	.....
43	6.1	.....	Tasseling	1.136	1.136	.....	.....
44	6.1	.....	Tasseled	0.000	1.176	.....	.....
45	20.5	.....	Tasseling	0.000	0.000	.....	.....
46	18.1	.....	Tasseling	0.909	0.909	.....	.....
47	29.9	.....	Tasseled	0.000	12.667	.....	.....
50	41.7	15.7	Tasseling	0.000	1.333	12	0
53	5.8	.....	Tasseling	2.083	6.666	.....	.....
55	32.1	.....	Tasseling	4.318	7.273	.....	.....
57	48.6	.....	Tasseling	0 000	2.500	.....	.....
69	14.4	.....	Tasseling	0.000	2.083	.....	.....
70	19.6	.....	Showing tassels	0.000	0.000	.....	.....
70	Check	.....		0.000	0.000	.....	.....
90	3.9	.....	Tasseling	0.000	0.000	.....	.....
93	18.2	.....	Not tasseling	12.083	17.917	.....	.....
95	30.4	.....	Tasseling	4.091	4.091	.....	.....
96	42.7	13.3	Tasseling	2.059	5.588	8	0
97	3.4	.....	Not tasseling	1.786	5.179	.....	.....
104	39.0	.....	Tasseling	1.250	7.500	.....	.....
107	20.4	2.2	Not tasseling	1.538	5.000	10	1
110	12.5	.....	Tasseling	1.250	1.750	.....	.....
111	25.5	.....	Tasseling	2.000	2.000	.....	.....
112	8.9	.....	Showing tassels	0.000	4 048	.....	.....
116*	36.5	3.7	Showing tassels	14.500	27.000	10	0
117	47.8	.....	Tasseling	0.500	10.250	.....	.....
118	52.4	5.5	Tasseling	0.714	4.464	21	1
120	15.8	7.6	Tasseling	2.272	9.545	6	0

Table 5—*Continued*

Line number (1930)	3- to 5-year average reaction		Stage when topped or detasseled August 24	Smut severity at end of 1932 growing season		Number ears bagged September 1	Bagged ears smutted end of growing season
	Total	Ear		Neck	Total		
122	44.7	6.5	Tasseling	6.250	14.583	5	0
125	47.0	5.5	Showing tassels	1.034	.345	6	0
126*	22.8	7.6	Tasseling	13.461	14.231	12	0
129	31.6	.....	Showing tassels	6.154	6.154	.....	.....
129	Check	.....		0.000	0.000	.....	.....
130	2.0	.....	Not tasseling	0.000	0.000	.....	.....
133	24.2	2.7	Showing tassels	0.625	0.625	24	0
133	Check	.....		0.000	0.000	.....	.....
135	65.9	21.8	Tasseling	7.593	36.296	13	5
138	12.2	.....	Tasseling	0.000	0.000	.....	.....
139	43.6	42.8	Tasseled	0.000	0.000	12	0
142	1.5	.....	Tasseling	0.000	0.000	.....	.....
143	21.6	.....	Tasseled	0.000	2.609	.....	.....
144	8.8	.....	Tasseled	0.000	4.737	.....	.....
147	16.6	4.0	Tasseling	1.176	4.706	6	0
148	25.7	.....	Tasseled	rot	1.599	.....	.....
149	14.1	.....	Tasseling	0.770	5.385	.....	.....
150	43.4	.....	Tasseled	0.000	0.000	.....	.....
151	30.5	4.9	Tasseled	0.000	0.625	5	0
152	7.1	.....	Tasseling	1.956	3.261	.....	.....
154	29.2	.....	Tasseling	7.059	17.647	.....	.....
154†	29.2	.....	Tasseled	1.177	7.353	.....	.....
155	28.4	7.6	Showing tassels	0.000	0.000	9	0
155	Check	.....		0.000	1.622	.....	.....
156	7.0	2.8	Showing tassels	6.500	11.000	22	0
156	Check	.....		0.000	0.000	.....	.....
157	4.7	.....	Showing tassels	0.000	2.778	.....	.....
161	2.6	.....	Tasseling	2.500	5.000	.....	.....
164	13.4	.....	Tasseled	0.000	8.888	.....	.....
166	10.5	7.5	Tasseling	0.000	0.000	12	0
166	Check	.....		0.000	1.667	.....	.....
167	26.6	.....	Tasseled	0.000	1.316	.....	.....
170	13.7	.....	Tasseling	2.885	2.885	.....	.....
172	10.3	.....	Tasseled	0.000	2.000	.....	.....
173	9.4	.....	Tasseling	0.000	.385	.....	.....
175	8.9	.....	Tasseled	0.000	0.000	.....	.....
176	40.8	.....	Tasseling	0.000	11.731	.....	.....
177	20.8	.....	Tasseled	0.000	13.929	.....	.....
178	17.6	.....	Showing tassels	0.000	4.722	.....	.....
180	19.6	.....	Tasseling	2.368	4.737	.....	.....
181	18.7	.....	Tasseling	1.000	3.500	.....	.....
182	15.2	.....	Tasseling	0.000	0.000	.....	.....
183	15.1	.....	Tasseled	0.000	5.526	.....	.....
185	23.3	.....	Tasseling	0.000	5.000	.....	.....
186	12.3	.....	Tasseling	0.833	1.250	.....	.....

* See Fig. 3.

† Planted July 2; detasseled August 27.

It is of interest to note (see Table 5) that topping at two stages of development was possible on S-154, a row of which was left standing because it was not needed for measurements taken on several series of these lines. It happened that the row in question was only 15 feet removed from the S-154 row of the topped series and had been planted

four days earlier than the latter. The tassels were pulled from this extra row late in the evening of August 27 and it showered lightly during the night. There were 17 stalks in each of these rows, and the conditions under which they grew were so nearly the same that the two are considered comparable. It may be said that the one was detasseled a week later than the other and developed only one-sixth as much neck smut.

6. Topping of F_1 Plants at Two Stages

The evidence from the detasseled selfed lines might be considered purely preliminary, and observational rather than experimental. However, the results seemed to indicate the importance of both the genotype and the stage of development of the host to smut increases called forth by topping. These points clearly warranted further study, and seed of a number of F_1's from practically homozygous selfed lines was available. Thus it was possible to test the effects of host genotype and stage of development on genetically uniform, vigorous populations in 1933, a rare opportunity.

One row each of 45 F_1 populations was planted June 2 and 3; the plants developed nicely, and, when they approached the tasseling stage, 180 to 200 each per F_1 were available for study. On July 10, immediately following a rain, every third plant was topped. On July 15, again following a rain, the second member of each set of three was topped. The third member of each set of three was left as check. By July 15, tassels had been exposed by nearly all the non-topped plants.

The results of this simple test of the effects of both genotype and time of injury are presented in Table 6, in terms of severity of smut. By the way of summary, it may be stated that:

In 42 out of 45 cases, smut was more severe on plants topped July 10 than on checks.

In 39 out of 45 cases, smut was more severe on plants topped July 10 than on plants topped July 15.

In 28 out of 45 cases, smut was more severe on plants topped July 15 than on checks.

In some cases, it was evident that topping, even on July 10, had not affected the amount of smut.

The increases in smut were principally at the nodes immediately below the points of severance of the tops or tassels. This fact is suggested by a comparison of the neck-smut severities with total severities in the data presented in Table 6.

These results show clearly that the genotype of the host is very important in determining reaction to smut after mutilation. Further, they show that, within a given genotype, the time of mutilation is all

important in determining smut reaction. This information may be of aid to corn breeder and corn producer alike, and its application is seemingly apparent.

Table 6.—The Severity of Smut on F_1 Populations from the Mating of Selfed Lines of Four Varieties of Corn, Showing the Effect of Topping at Two Stages

Selfed-line matings by varieties	Date of topping and smut severity					
	July 10		July 15		Check*	
	Total	Neck	Total	Neck	Total	Neck
Rustler						
15x17	7.58	0.73	3.05	0.00	4.24	0.00
15x18	5.00	1.23	8.77	3.60	3.52	0.18
15x20	4.83	0.93	0.58	0.58	0.00	0.00
15x24	1.77	1.61	0.87	0.71	1.05	0.16
15x25	4.09	2.73	3.47	1.94	0.37	0.00
15x26	2.92	0.17	3.33	0.00	0.18	0.00
16x17	8.54	3.23	5.70	0.90	7.45	0.00
16x20	5.85	2.29	3.36	0.45	1.61	0.00
16x21	6.92	1.16	2.56	0.31	3.68	0.00
16x25	3.52	0.19	4.58	1.04	1.79	0.19
16x26	7.33	3.53	4.27	2.18	5.43	0.00
16x29	2.28	1.50	1.24	0.53	1.68	0.92
17x20	4.44	1.35	1.88	0.16	1.27	0.00
17x21	17.38	5.95	15.00	7.16	9.18	0.49
17x24	9.81	5.77	8.06	4.39	5.34	0.22
17x26	10.50	5.83	9.66	4.22	9.62	1.51
17x29	15.27	10.82	5.57	1.97	1.11	0.37
18x23	3.56	2.73	3.86	0.98	2.46	0.00
18x24	8.21	4.25	2.40	0.67	5.94	0.52
18x25	9.75	6.27	7.95	3.21	2.76	0.00
20x21	2.66	0.00	2.62	0.77	0.98	0.00
20x23	1.67	0.94	0.16	0.16	0.32	0.00
20x25	3.59	1.54	2.79	2.09	2.56	0.00
20x26	1.33	1.33	1.00	0.17	0.35	0.00
20x29	7.42	0.50	1.50	0.00	0.00	0.00
Minn. No. 13						
1x2	10.00	5.91	0.55	0.36	0.00	0.00
1x4	12.19	8.96	4.13	2.79	2.02	1.63
1x7	14.83	5.25	3.00	1.46	1.33	1.16
1x9	6.37	1.57	1.15	1.15	0.20	0.20
1x13	6.61	2.18	0.80	0.45	0.71	0.18
2x5	4.90	0.83	2.28	0.00	2.44	0.22
2x7	4.68	1.53	3.17	3.02	4.92	4.10
2x9	2.27	0.45	0.18	0.18	1.32	0.88
2x10	8.95	7.63	1.61	0.40	0.97	0.97
2x11	6.16	5.54	0.69	0.34	0.00	0.00
4x5	7.77	3.83	2.98	0.64	5.11	3.22
4x8	3.27	1.16	1.49	0.35	0.87	0.00
Minn. No. 23						
31x35	3.63	1.85	2.61	0.63	2.95	0.57
31x37	6.76	2.68	8.31	2.36	10.36	4.35
34x37	5.56	0.48	5.38	1.46	5.92	1.00
Golden Bantam						
38x50	4.72	3.24	1.43	0.33	1.94	0.13
40x46	4.38	1.51	1.68	0.36	1.42	0.23
41x42	1.10	0.85	0.98	0.22	0.21	0.00
41x43	1.21	1.00	0.36	0.10	0.70	0.26
41x49	0.70	0.17	1.22	0.09	0.15	0.00

* Total partially corrected for comparison with topped plants by subtracting tassel-smut severities. The latter were, for practical purposes, negligible.

7. The Effects of a Wind and Rain Storm

In 1932, during the first half of the summer, weather conditions were practically ideal for consistent rapid growth of corn planted on May 30, but, contrary to what might have been expected because of their succulence, very few of the plants developed early-season smut. On July 9, Northwestern Dent of this planting was tasseling, but Rustler was still in the spiral stage, between 20 and 24 inches tall. There was a fairly heavy wind and rain storm in the evening of that day, and it was obvious the next morning that the twisting of the plants in the wind and beating of the rain had caused meteoric water, well supplied with soil, pollen, etc., to sink lower in the leaf rolls of Rustler than it would in a gentle rain. During the next week the corn unrolled very rapidly, and, since there were no rains, the level to which the storm had driven extraneous material was well marked on the growing leaves by bands of dried soil that made it easy to follow the development of smut in particular tissues which would hardly have been reached by inoculum except for the storm's effects.

Numerous flecks were apparent in and around the dirt bands on July 12, and later many of them became necrotic. On July 15 and 16 a microscopic examination was made, after vital staining, and germinated smut spores and mycelium, quite typical of corn smut, were found in the chlorotic and necrotic spots. Many of the flecks were caused by *Puccinia sorghi* and perhaps other fungi. Most of the infections were abortive, advancing no further than the necrotic spot stage; some formed tiny wart-like galls, and a few produced fairly large galls that persisted until the end of the season. It was very evident that the younger the marked leaves, the greater the smut development; and on many plants the leaves above the last dirt-marked leaf were quite severely smutted.

8. The Relation of Early-season Leaf Smut to Late-season Nodal-bud Smut

It is generally agreed (27, 28) that the so-called nodal buds or potential-ear shoots are the most commonly smutted organs of the corn plant. Just when they become infected, however, has not been known. Platz (27) and the writer (38) failed to induce increases in the prevalence of smut by dropping inoculum between leaf-sheaths and stalks. Therefore it seems that lack of inoculum after the plant is unrolled does not limit the amount of shoot smut. It appeared that inoculation during the leaf-spiral stage might account for this smut and that a study of the relation of the early-season leaf smut to the late-season smut subtended by these leaves might help to elucidate the question.

In the Rustler checks of the June 15 planting in 1932 each stalk that developed leaf smut between the one-foot and the tasseling stages was

tagged with a record of the number of leaves smutted. There was a severe loss of tags because of the drying and breaking of leaves, but at the end of the season a total of 229 tagged plants, distributed through four series, were available for observation. The prevalence and severity of the subtended smut, i.e. smut at nodes (including necks and shoots on the tagged plants), was compared with the prevalence and severity of the subtended smut on all the stalks of the plots, i.e. total subtended smut. The part was best compared with the whole in this case, because it could not be said that all stalks that were not tagged did not have early-season leaf smut. The results of the comparisons were as follows:

1. Severity of subtended smut:
   Tagged 15.542 ± .4229
   Total 9.915 ± .6384
   Difference 5.627 ± .7658 in favor of tagged
2. Percentage of plants with subtended smut:
   Tagged 49.46 ± 2.5396
   Total 37.49 ± 2.3430
   Difference 11.97 ± 3.4554 in favor of tagged

It is clear from these figures that Rustler plants which developed leaf smut early in the season were more likely to bear nodal-bud smut later in the season than stalks which did not have early-season leaf smut. The heterozygosity of normal variety corn, more specifically the range of genotypes found in a population of corn plants of a variety such as Rustler, may be considered an important factor in this result. It must be remembered, moreover, that subtended smut occurs on a goodly percentage of plants on which it is impossible to find early-season leaf smut and that the major portion of nodal-bud smut is yet unaccounted for (see Table 8 and remarks on p. 37). Inoculum might easily reach the potential-ear shoots of corn still in the spiral stage without producing galls on the leaves.

It is of interest to note, in passing, that the severity of subtended smut on June 15 Rustler is 9.915 in comparison with a total severity of 10.291, indicating the major importance of this type of smut on Rustler.

9. Results of Bagging Selfed-line Shoots

Kyle (20) has reported a correlation between the tightness of husk covering and resistance to ear smut, and it is well known that ear tips are commonly smutted in some varieties and lines and not in others. It is also well known that individual smut kernels, highly suggestive of silk infection, are sometimes found well protected in otherwise healthy ears. In Table 5 the results of detasseling selfed lines are given to-

gether with additional information concerning the infection of ears by *Ustilago zeae*. On September 1, 1932, a total of 203 shoots on 18 of the lines of the detasseled group were bagged for the purpose of noting the effects of the artificial protection of silks on the development of ear smut. Shoots which had not yet exposed their silks were carefully selected and were covered with parchment-paper bags just as they are covered in controlled pollination studies. The bags were not removed as they are when pollen is to be applied to the silks, however, and when notes were taken at the end of the growing season there was no evidence whatever of silk infection on any of the 203 ears. However, 7 of the 203 ears, excepting the husks, were completely transformed by smut, 5 of them occurring in 13 bagged ears of S-135. That the shoot husks would have been smutted if infection had taken place in the young bud stage seems a fair assumption. The infections resulting in these 7 smutted ears had quite certainly taken place either through the young husks before bagging, or, still earlier, through the nearby leaf or stalk tissue and had proceeded into the shoots from the nodes after the husks were well developed. S-135 afforded excellent observational evidence on this point. On many of the smutted, non-bagged ears found on 27 stalks of this line tiny wart-like galls in the husks clearly marked the course of the smut from the outside to the completely smutted cob and kernel tissues within. S-135 is the only line or variety on which this has been observed by the writer, but it seems fairly good evidence that infections through the tender coverings of young shoots cause ear smut in this line and perhaps in others in which the symptoms are not so clear.

10. Results of Repeated Dusting with Chlamydospores

Observations and counts of leaf and tassel smut were made on the selfed lines grown in the smut plots (16) at University Farm on July 8, 1932, when the lines ranged in height from 10 to 28 inches, and again on July 21 and 22, when nearly all the lines had tasseled. Beginning on June 24, chlamydospores had been dusted over each plant of these plots once a week. On July 8 there was very little smut, but on July 21 and 22 considerable leaf and tassel smut was found, and it was judged that part of the smut in some lines was traceable to the heavy dusting with chlamydospores, perhaps in combination with the effects of a wind and rain storm on July 9.

A plot of two rows each of Rustler and Northwestern Dent planted on June 30 was available, and it was used to test the effect of dusting with chlamydospores at four-day intervals from the eight-inch stage to tasseling. Late in the evenings, after the dew had formed and the

breezes had died down, dry chlamydospores collected from early galls of nearby early-planting borders were dusted over each plant of the inside rows of the plot, leaving the outside rows of the four-row strip as checks. There was, of course, some drifting of the chlamydospores, but it is a conservative estimate that plants of the check rows did not receive one one-thousandth as many spores as those which were dusted directly. The results of this test, presented in Table 7, show quite clearly that the application of large numbers of chlamydospores to the spiral heads of these plants did not increase the severity of smut. Rather than an increase, a slight decrease, the significance of which is doubtful, was noted.

This test differed from that reported for the June 15 and 30 corn in Table 3, treatment 3, in that spores were applied at four-day intervals from the eight-inch to the tasseling stage, whereas in the previously discussed experiment the dusting was done at only one time, namely, during lulls in the showering on July 25. In view of observations presented on page 32 and these results, we must, for the present, assume that any effects of heavy dusting with chlamydospores on the selfed lines in the nursery came through the coincidence of the storm of July 9 and the proper stage of growth of the affected lines.

Table 7.—The Results of Heavily Dusting Rustler and Northwestern Dent Corn with Chlamydospores at Four-day Intervals from the Eight-inch Stage to Tasseling

Treatment	Rustler		Northwestern Dent	
	Number of stalks	Smut severity	Number of stalks	Smut severity
Dusted rows	212	4.685	198	6.465
Check rows	215	6.023	198	7.248
Series check*	312	7.388	318	6.934

* Figures for nearby plots used in "date of planting" study. See Table 19.

From these results and those presented in Tables 3 and 4 it must be concluded that the application of inoculum to the leaf-spirals fails to increase the amount of smut. This is the same result as that obtained earlier by Griffiths (9), Platz (27), and the writer (38). The repeated failure to increase the severity of smut above natural infection by applications of spores and sporidia to spiral heads and nodal buds can hardly be taken to mean anything else than a sufficiency of inoculum in nature to give the greatest smut development other conditions would allow. We can not do otherwise than conclude that smut reaction under the conditions of these studies was largely a question of effectiveness of inoculum already present rather than one of inoculum supplied.

11. A Review of Results Obtained by Inoculating with the Hypodermic Syringe

After reviewing the results (6, 9, 15, 16, 27, 30, 36, 38, 39) of inoculation methods and injury practices involving the needle and syringe the following conclusions seemed justified:

1. Puncturing[4] the growing tip and other smut-susceptible tissues with the needle of the syringe does not increase the prevalence of corn smut (see page 15, a résumé of 1931 results).

2. Forcing dry chlamydospores into the tender leaf-spiral tissues by means of the hypodermic syringe results in heavy smut on a high percentage of plants (39).

3. Injection of sterilized water with a hypodermic syringe results in heavy smut, the later the injection (stage of plant remaining the same), the higher the percentage of smutted plants.

4. Injecting a suspension of sporidia or chlamydospores results in heavy smut, frequently accompanied by severe necrosis.

It seems fair to consider that the puncturing with a dry needle should have given an increase in smut if the *modus operandi* of the injection of sterilized water were to be considered the carrying inward of spores from the outside of the plant. Statement 2 is evidence that introduced water was not necessary to infection. In view of the facts presented in statements 1, 2, and 4, it seems that the most reasonable interpretation of the role of sterilized water is the following:

> The injected liquid furnishes an avenue or connection between the inoculum in the meteoric water standing in the upper leaf-spiral and the susceptible tissues lower in the leaf roll (see Fig. 1).
>
> The greater the amount of inoculum the more often a column of liquid would function in allowing the smut to sink to the level of susceptible tissues. Therefore increased effectiveness of the method with the advance of the season would be expected because of the tremendous increase of inoculum under conditions favorable for smut.

If this interpretation of the effects of injecting sterilized water is correct, the results given by the practice are indicative that inoculum may be deficient and a limiting factor in smut prevalence early in the season. When considered in connection with the above interpretation, however, these same results, marked increases above natural infection, can only mean that on later corn the natural prevalence of smut at University Farm was limited by the gross morphology and growth responses of corn during the leaf-spiral and later stages, because it may be safely assumed that during the latter half of the growing season practically every plant was supplied with abundant natural inoculum.

[4] It was noted during August, 1932, that injury by the larvae of the western corn root worm, *Diabrotica longicornis* Say., to many plants of several different selfed lines meant no increase in smut. A number of plants which were bored through and through by the larvae were examined carefully, but not one was found with smut galls relatable to the tunnels.

12. The Influence of Injecting Sterilized Water on the Location of Smut Galls on Corn Inoculated at Different Stages of Development

It is suggested by the preceding discussion of results of injecting sterilized water that comparisons of the locations of galls on plants thus inoculated with the locations of galls due to natural inoculation may yield good evidence on the time of infection of certain organs of the plant, especially nodal-buds. This question was discussed at some length in Section I-8, pages 32 and 33. A good comparison of stages of development at inoculation is allowed by the June 15 and June 30, 1932, plantings treated July 25. In the absence of complete records of the location of galls on the June 15 checks, it seems that the results of treatment 7 (see Table 3), the application of a small amount of soil to the spirals, a very natural method which has not affected the amount of smut, may fairly be substituted for the checks in a comparison with the results from injecting sterilized water. The results of these two treatments on Rustler are given in Table 8.

It is apparent from the data in Table 8 that, other than the expected greater amount of leaf smut on the younger corn, there is no important difference in the location of smut on the two plantings under treatment 7 (or natural inoculation). Injection of sterilized water has, however, markedly affected the location of smut, and the points of greatest increase differ with the plantings. On corn inoculated at the 10- to 12-inch stage (June 30 planting) there is four times as much stalk smut as on that inoculated at the 20- to 24-inch stage (June 15 planting). On the latter there has been a marked increase in tassel smut, but on the younger corn the amount of tassel smut has not been altered. There has been a greater increase in leaf and neck smut on the younger corn than on that of the June 15 planting.

Table 8.—The Severity of Smut at Different Locations on Rustler Corn Planted June 15 and June 30, 1932, and Treated by the Injection of Sterilized Water or the Dropping of Soil Into Leaf-spirals

Gall location	June 15 planting		June 30 planting	
	Sterile H_2O injected	Soil dropped	Sterile H_2O injected	Soil dropped
Leaves	2.492	0.231	5.992	1.612
Base*	1.100	0.000	0.201	0.076
Nodal buds	7.218 ± .542	6.618 ± .354	7.044 ± 1.226	5.407 ± .718
Ears	0.000	1.156	1.049	1.178
Stalks	2.526	0.732	10.017	0.323
Neck	1.284	0.687	2.280	0.368
Tassel	4.145	0.104	0.187	0.232
Total†	18.745	9.528	26.770	9.196

* Brace root and below-ground shoot galls.

† Failure to check with means of Table 3 is due to carrying too few decimal places.

The most striking fact presented by the figures in Table 8 is the constancy of the amount of nodal-bud smut, regardless of treatment or planting. There is no significant difference between any pair of the nodal-bud smut severities. This is all the more remarkable when it is remembered that the June 30 planting was inoculated at the 10- to 12-inch stage. It seems to the writer that this fact can only mean that the development of nodal-bud smut, by far the most important type of smut on Rustler, is limited by factors other than inoculation. It is strongly suggestive that, even if infection does occur when the plant is young, the development of shoot or nodal-bud smut awaits the operation of other factors. This is in agreement with field observations on the appearance of nodal-bud smut. Very few galls develop on Rustler before the plants have either grown to full size or have been arrested in growth and caused to mature and harden by unfavorable conditions.

EXPERIMENTAL SECTION II

The Relation of Host Vigor, Influenced by Ecological Factors and Cultural Practices, to the Development of Corn Smut

In 1930 Kyle (21, p. 231) aroused considerable interest in the relation of host vigor to the development of corn smut by summarizing extensive studies on the agronomic phases of the problem as follows: "The data presented show a clearly defined tendency for vigor to be directly associated with the relative number of smutted potential ear shoots. This association holds when the differences in vigor are caused *(a)* by differences in the environment, *(b)* by plant variations within crosses, *(c)* by differences in heterosis between crosses and their selfed parents, and *(d)* by differences inherent in the different selfed lines. In selecting smut-resistant selfed lines, it must be borne in mind that smut resistance in some cases may be due to lack of vigor. The use of strains having such low vigor may result in lower yields."

Mazé and Mazé (23), in 1932, interpreted the relation of host vigor to smut development as the reverse or direct opposite of that outlined by Kyle. They summarized 30 years' study of the influence of manuring and fertilization by stating that corn which grew rapidly and consistently, i.e. without the common interruptions of growth due to unfavorable soil and weather conditions, developed less smut than corn that grew under less favorable environmental conditions.

That such discrepancies as the above are common in the literature dealing with the ecological relations of the disease is shown by the brief résumé presented earlier in this bulletin. Many of the conflicting results are undoubtedly due to the differences in the areas for which the par-

ticular observations apply. The following experiments and observations were made in an attempt to clarify the situation.

1. Influence of Soil Moisture Within One Check Plot in 1931

During the summer of 1931 the writer had opportunity to make several interesting observations on the relationship between soil moisture and the development of smut. These observations were allowed by the topographic irregularity of the field used for inoculation, cultivation, and date-of-planting studies. This so increased the experimental error of certain tests that it paled or wiped out significant differences, but it is the writer's opinion that the incidental observations are perhaps as valuable as more clear-cut results might have been. A diagrammatic representation of the contour of the field is presented in Figure 4, and reference to it will greatly facilitate explanation.

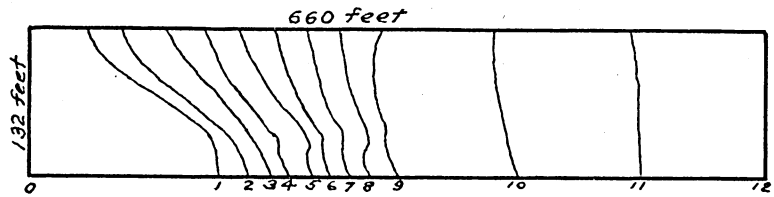

Fig. 4. Contour diagram of University Farm field A8 used for corn smut studies, 1930 to 1932 inclusive, the interval representing approximately six inches.

The check plot (usual cult.) of the cultivation series 4 (see Section II-6, Table 14), planted May 30, was located so that from east to west the rows, two each of Rustler and Northwestern Dent, extended from the 0 level to the 3 level as represented in Figure 4. Having endured intense heat from July 4 to July 28 without rain, the Rustler had been retarded in the late leaf-spiral stage, but Northwestern Dent had tasseled before the severe drouth checked its growth. Counts for the seasonal increase of smut were made on July 31 (see Table 18), and it was observed that smut was most severe on the Northwestern Dent in the low area which had remained moist throughout the drouth. On the Rustler, however, there was very little smut on the plants that had grown a little each day on moist soil of the 0 area and very little on the plants of the higher one-third, where the corn had wilted so severely that it had not recovered sufficiently three days after a good rain to start growth again. However, on the Rustler in the half-way-between area, where the plants had wilted during the day and regained turgidity at night during the drouth, growth was being resumed, and the new leaves of nearly every plant were smutted. The contrast was so striking that detailed analysis seemed entirely unnecessary. The difference per-

sisted to the end of the season and furnished a striking example of the indirect influence of soil moisture on smut development through the retardation of the development of corn in the leaf-spiral stage. It also suggests that wilting either facilitates entrance and advance of the smut fungus or perhaps allows inoculum to move to lower levels in the leaf spirals, where tissues are more tender. Another possible explanation of this result is that the delayed growth of the plant allows smut hyphae to overtake cells that might otherwise not be reached, the spread at the particular stage of the plant meaning the involvement of more portions of the unrolled plant.

2. Comparison of Series Under Different Growth Conditions, June 15 Planting, 1932

In 1932 the irregularity of the field again afforded an opportunity to observe the effects of soil moisture on the development of smut on the two varieties used in the date-of-planting studies. Referring to the diagram of the field presented in Figure 4, it may be said that series 3 of June 15 planting was at the 5 to 7 level on the steepest portion of the slope and series 4 was at the 0 level. Series 3, on the slope, was severely stunted by drouth between July 27 and the middle of August, when Rustler was in the tasseling stage, while series 4, on the low moist soil, made good growth daily and was rank at the end of the season. A summary of the prevalence and severity of smut on these two plots is presented in Table 9, which also serves as a comparison of the severity and prevalence methods of noting corn smut. The average heights of 100 stalks of Rustler from each of series 3 and 4 were, respectively, 55.595 ± .1868 and 78.15 ± .5352 inches.

This seems again a clear-cut illustration of the importance of variety and stage of development to environmental response of smut and shows that smut development and vigor of plant are not necessarily correlated. The response of Rustler in this instance is an exception to Kyle's generalization (21), "Vigor and smut susceptibility are directly associated."

Table 9.—The Prevalence and Severity* of Corn Smut on Rustler and Northwestern Dent Under Conditions Causing Premature Ripening in Comparison with Conditions Favoring Rank Growth

Variety	Series 3—premature ripening			Series 4—rank growth		
	Number of stalks	Smut prevalence	Severity	Number of stalks	Smut prevalence	Severity
Rustler	394	38.1	10.2	367	35.0	9.8
Northwestern Dent	251	44.6	9.6	348	50.0	12.9

* Prevalence = the percentage of stalks smutted. Severity = the calculated percentage reduction in yield due to the smut galls found.

3. Results of Fertilizer Test in 1931

In 1931, a very dry season, a randomized block test of manure and commercial fertilizers was made on a field that had been cropped to corn for several consecutive years. The last two crops had been grown since stable manure had been applied. Plots of three 40-foot rows each of Rustler and Northwestern Dent, thus approximating 1/60 acre, were treated as follows in quadruplicate series:

1. Check—no treatment
2. K—2.5 pounds muriate of potash
3. P+K—2.5 pounds muriate of potash plus 3.5 pounds treble superphosphate
4. N+P+K—4 pounds sodium nitrate, 3.5 pounds treble superphosphate, 3.5 pounds muriate of potash
5. Check 2—no treatment
6. P—3.5 pounds treble superphosphate
7. M+N+P+K—manure at 10 tons per acre, plus fertilizers as given in number 4 above
8. M—manure at 10 tons per acre
9. Check 3—no treatment
10. N—4 pounds sodium nitrate
11. N+P—4 pounds sodium nitrate plus 3.5 pounds treble superphosphate
12. N+K—4 pounds sodium nitrate plus 2.5 pounds muriate of potash

The fertilizers and manure were mixed in the soil May 27, and the corn was planted May 29 and 30. Only two pounds of sodium nitrate were applied to each N plot at this time; the remaining two pounds per plot were spread July 25. Because of the severe drouth, no striking effects of the fertilizers were to be observed.

Counts giving the prevalence of smut were taken on this corn August 29 and 31, and the results have been analyzed by the variance method (7). The analysis indicates that there were no differences due to treatments on Rustler but that treatments affected the prevalence of smut on Northwestern Dent, differences of 3.5 between means being expected by chance only once in 20 trials. The results for both varieties are summarized in Table 10.

It is interesting to note that on Northwestern Dent, N, which in greenhouse tests (30, 37) of plants inoculated by the syringe method renders the plants more susceptible to smut, has actually reduced the smut prevalence below that of the checks, while M+N+P+K and N+P are the only treatments that have given significant increases above the checks. The effects of these treatments on observable characters of the plants were slight, the N inducing more rapid growth during June and the latter two treatments causing slightly earlier drying than in surrounding plots. These interesting results were, without doubt, partly due to unusual weather conditions, and they are possibly most important

as a reminder that fertilizers must be tested for several years before one can properly evaluate the factors involved in their effects.

Table 10.—The Mean Prevalence of Smut on Rustler and Northwestern Dent Corn Under Different Fertilizer Treatments, 1931

Treatment	Rustler*	Northwestern Dent†
1. Check 1	42.1	46.5
2. K	42.2	48.7
3. P+K	41.7	47.3
4. N+P+K	40.5	49.8
5. Check 2	31.8	47.0
6. P	37.3	48.8
7. M+N+P+K	41.4	51.3
8. M	38.0	48.2
9. Check 3	38.5	46.4
10. N	40.5	41.4
11. N+P	41.0	50.1
12. N+K	41.4	48.5

* No difference due to treatments.
† Differences of 3.5 may be considered significant.

4. Results with Fertilizers in 1932

The plots used for fertilizer tests in 1931 were not available in 1932. The only available land was a field, already planted to Rustler on May 26, which had grown corn and had been manured regularly for several years. Rather than discontinue the fertilizer and manure tests, the following treatments were applied to 12 x 12 row plots in quadruplicate randomized series on June 3, as the corn was emerging:

1. N+P+K—10 pounds sodium nitrate, 8¾ pounds treble superphosphate, 6¼ pounds muriate of potash
2. P+K—8¾ pounds treble superphosphate, 6¼ pounds muriate of potash
3. N+K—10 pounds sodium nitrate, 6¼ pounds muriate of potash
4. N+P—10 pounds sodium nitrate, 8¾ pounds treble superphosphate
5. Check 1—no treatment
6. K—6¼ pounds muriate of potash
7. P—8¾ pounds treble superphosphate
8. N—10 pounds sodium nitrate
9. M—manure at 12½ tons per acre
10. Check 2—no treatment

An immediate deep cultivation mixed the materials in the soil.

This corn was favored by unusually good growing conditions during the first half of the season, and it developed very little smut. Drouth and excessive heat during August did not permit normal maturity, however, and caused premature drying in certain spots of the field. Notes that allowed calculation of the severity of smut were taken September 7 to 9, inclusive, and the data, which have been analyzed by Fisher's method, are presented in Table 11. The analysis indicates that there

are no differences due to treatments in this experiment, but this does not mean that under other circumstances fertilizers and manure would not markedly affect the smut development.

Table 11.—The Severity of Smut on Rustler Corn Under Different Commercial Fertilizer Treatments, Thompson Field, 1932

Treatment	Series*				Mean
	1	2	3	4	
1. N+P+K	3.599	4.433	3.955	3.357	3.836
2. P+K	3.478	2.342	3.029	2.930	2.945
3. N+K	3.388	2.922	3.462	3.352	3.287
4. N+P	2.912	3.752	3.226	4.286	3.544
5. Check 1	5.293	3.883	2.830	3.151	3.789
6. K	4.344	3.292	2.530	3.691	3.464
7. P	2.936	2.724	3.262	3.774	3.124
8. N†	4.488	3.175	3.297	3.307	3.567
9. M	3.011	2.488	3.339	3.333	3.043
10. Check 2	3.400	3.826	3.268	3.684	3.545

* Plots contained 500 to 700 stalks each and were about 1/25 acre in area.
† Two applications of nitrate of soda, 5 pounds June 3 and 5 pounds July 19.

5. The Manure-Chlamydospore Tests

In 1932 one strip of Thompson field, the location of the commercial fertilizer test described above, was devoted to a Latin-Square (7, 10) trial of the effects of manure and smut spores, applied to the soil separately and together, on the development of corn smut. Plots 7 x 20 rows, approximately 1/25 acre in area, were given the following treatments in Latin-Square order:

A. Manure—12½ tons per acre
B. Manure—12½ tons per acre plus smut spores
C. Smut spores only
D. Check—no treatment

The plots were laid out and the manure was applied June 4 when the corn was two inches tall. Smut, collected from the 1931 smut plots and stored in a dry place during the winter, was applied to the B and C plots while rain was falling on June 6 and again on July 9. About one peck of smut was scattered over each plot per application, so it does not seem that there could have been a shortage of soil inoculum in this test. The soil was tilled as soon after each application as it had dried sufficiently so that puddling would not be excessive.

Notes which allowed calculation of the severity of smut were taken September 7, and the results are presented in Latin-Square form in Table 12 to show the distribution of smut by plots. The Latin-Square method of field-plot technic gives lower error than any other method, but the analysis of the data shows that treatments have had little if any-

thing to do with the differences between the plots, for the variance due to error is larger than that due to treatments. It seems quite clear that other factors than the amount of inoculum limited the development of smut on these plots, and under other growth conditions the results might have been entirely different.

Table 12.—Results of "Latin-Square" Manure-Smut-Spore Test on Thompson Field, with Rustler Variety, 1932

Treatments* and smut severity				Treatment means
A†—2.595	C—3.158	B—3.577	D—3.535	A—3.074
B —3.656	D—3.750	C—2.932	A—2.816	B—3.389
C —2.454	A—3.182	D—2.755	B—2.789	C—3.016
D —3.208	B—3.532	A—3.703	C—3.519	D—3.312

* For treatments refer to list above.
† 475 to 624 stalks per plot.

Rustler grew much more vigorously on Thompson field than on field A 8 in 1932, the former being unquestionably the more fertile land. It is shown by a comparison of the data presented in Table 19 (for the May plantings) with those of Tables 11 and 12 that there was less smut on Thompson field than on field A 8 in this one season. The plants of Thompson field grew very rapidly throughout the leaf-spiral stage. On July 6 when the plants were approaching the tasseling stage, counts of smut on the inside border rows revealed that only 14 out of 1,688 stalks had developed early-season leaf smut. Thus, practically all of the smut galls recorded for the Rustler on Thompson field were nodal-bud or potential-ear galls, and these appeared after the stalks had reached full size.

The manure-chlamydospore test was repeated in Latin-Square form in 1933 on "Schacht field." Normal Rustler was planted June 1, and on the next day the manure was spread over the areas to which it was allotted, the plots being 11 x 12 rows in extent. The seedlings were emerging on June 7, but not until June 20, when they were 6 to 8 inches tall, did there occur a steady rain which would make application of the spores safe. There were only enough spores of the 1932 collection to allow ½ peck per plot, but at the one application a peck of 1931 collection was scattered over each smut-spore plot. The viability of these spores of both collections was ascertained by laboratory germination tests.

In this trial in 1933 the effects of the manure on the vigor of the corn were satisfactory, i.e. there was a clear-cut difference in the vigor of the plants on the manured and non-manured plots. Notes allowing the calculation of the severity of smut were taken at the end of the growing season, and the data are presented in Latin-Square form in Table 13.

Regardless of the distinct increase of vigor due to the manure, an analysis of variance of the data of Table 13 shows that the variance due to error is larger than that due to the treatments, the same results as those obtained in 1932.

Table 13.—Results of "Latin-Square" Manure-Smut-Spore Test on Schacht Field, with Rustler Variety, 1932

Treatments* and smut severity				Treatment means
A—9.66	C—6.40	B—5.84	D—7.54	A—6.84
B—7.60	D—6.71	C—5.58	A—6.41	B—5.62
C—4.00	A—4.10	D—5.96	B—5.41	C—4.83
D—4.89	B—3.62	A—7.18	C—3.35	D—6.28

* See p. 43 for the list of treatments.

6. The Effects of Marked Differences in Vigor Resulting from Cultivation Practices in 1931

In 1931 a Latin-Square test of the effects of four cultivation methods was carried out on plots consisting of two 135-foot rows each of Rustler and Northwestern Dent planted May 30. The methods tested were:

A. Cultivation often and deep—deep regularly and twice more than usual
B. Check—usual cultivation for University Farm
C. No cultivation—weeds scraped off with hoe
D. Listed—ridges plowed down after 72 days

Owing to the dry weather, perhaps, the influences of these cultivation practices on the vigor of the corn were remarkable. The effects of the treatments appeared early and became more marked as the season progressed. The plants on plots cultivated often and deep were the most vigorous and made fairly good daily growth throughout the season; those cultivated as usual for University Farm ranked next in vigor; and the non-cultivated and listed plots ranked in descending order. It may be said, figuratively, altho no measurements were taken, that these plots formed 8- to 10-inch stair-steps in the order mentioned and that stalk diameter was proportional to height. The prevalence of smut on these plots cultivated by different methods was noted at the end of the growing season, and the data are summarized in Table 14.

Application of analysis of variance procedure (7, 10) to the individual data from which the means of Table 14 are derived shows that only in Rustler are there significant differences due to the cultivation practices. It is interesting to note from the data in Table 14 that for Rustler there are, on the basis of the differences necessary for significance, two levels of smut prevalence. Corn cultivated as usual for University Farm and that not cultivated had more smut than the corn cultivated often and deep, which was the most vigorous, or that listed,

which was the least vigorous. The close relationship between nodal-bud smut and total smut prevalence on Rustler is also striking, but no such relationship holds for the Northwestern Dent strain.

Table 14.—The Mean Prevalence of Corn Smut on Rustler and Northwestern Dent Cultivated in Different Ways, 1931

Cultivation practices	Total smut		At nodal buds only	
	Rustler*	Northwestern Dent	Rustler†	Northwestern Dent
A. Often and deep	43.2	52.3	26.8	18.2 ± .875
B. Usual (U. F.)	51.7	58.2	32.9	23.3 ± .580
C. None	54.9	51.9	36.4	24.3 ± .697
D. Listed	42.2	56.9	27.4	25.8 ± 1.448

* 7.2 may be considered significant difference between means.
† 4.8 may be considered significant difference between means.

It is noteworthy that the prevalence of nodal-bud smut on Northwestern Dent is in inverse relation to the vigor of the corn, while the total prevalence shows no relation to vigor. Since it was evident that the Northwestern Dent cultivated often and deep had less nodal-bud smut than that under the other three treatments, separate probable errors were calculated for these means. Calculation of the probable errors of the differences between the means and checking of odds supports the conclusion that there was less nodal-bud smut on Northwestern Dent cultivated often and deep than under any of the other three treatments, not one of which induced significant differences in the amount of nodal-bud smut. In this experiment, therefore, there was less nodal-bud smut on vigorous plants of both varieties than on weaker plants.

It is clearly shown by the results presented above that the two varieties, widely different in other respects, also differ in reaction to smut under different conditions of vigor. On neither variety, however, is the prevalence of smut directly proportional to the vigor of the plants, and on Rustler there is definitely an inverse relation. Listed corn was so severely stunted that it was apparently below the level of vigor necessary for the production of smut galls, which are products of combined efforts of host and parasite. From these data it is evident that vigor of host is not always associated with smut susceptibility, and that the opposite is true for certain varieties under certain conditions.

Observations (see pp. 39 and 40) during the latter part of July and early August indicated that the stage of development that the plants had reached before the critical drouth period, which lasted from July 5 to July 28, was very important in determining the responses following the rain of July 28. It was noted on July 30 that the listed Rustler, whose development had been arrested in the late spiral stage by the drouth, was beginning to push out leaves again, whereas the plants on the non-

cultivated and listed plots of Northwestern Dent, halted in the tasseling stage, were showing no signs of renewed main-stalk growth. The subsequent development of galls on the latter corn was almost entirely in the laterals, which generally grow more rapidly when development of the main stalk has ceased. The listed Rustler grew out of the spiral stage with unusually high percentages of leaf and tassel smut, but dry weather and excessive heat again stopped its growth entirely, and it failed to produce the great amount of nodal-bud or shoot smut found in non- and usual-cultivation plots.

7. Cultivation Methods in 1932

In 1932 a test of cultivation methods was carried on much the same as in 1931, but an additional treatment, the leaving of weeds to compete with the corn, was included. Unfortunately, the corn was planted May 30 and subsequent weather conditions were perhaps as favorable for rapid development as could be expected in Minnesota. Unfortunately again, the soil of the "weed" and "no cultivation" plots was stirred during the week following planting, on the assumption that one light cultivation was desirable to enable the seedlings to get a start. The growth was so rapid that not even the plants in the "weed" plots were checked before they had reached the tasseling stage. Other than the small effects of late-season competition by weeds, there were no evident differences in the vigor of plants in the different plots. Analyses of variance of the results, which are presented in Tables 15 and 16, indicate that there were no differences in severity of smut due to different methods of cultivation.

Table 15.—The Severity of Smut on Rustler Under Different Methods of Cultivation, 1932

Cultivation	Series				Mean
	1	2	3	4	
Weeds	6.510	6.649	4.821	4.167	5.537
Usual (U. F.)	5.285	5.548	5.600	3.308	4.937
None	5.433	5.302	4.839	5.675	5.312
Listed	4.530	6.298	2.945	2.416	4.047
Often and deep	5.878	6.902	5.225	2.038	5.011

Table 16.—The Severity of Smut on Northwestern Dent Under Different Methods of Cultivation, 1932

Cultivation	Series				Mean
	1	2	3	4	
Weeds	8.020	5.848	5.153	5.900	6.230
Usual (U. F.)	8.050	8.215	6.963	9.204	8.108
None	8.682	5.000	4.742	13.333	7.939
Listed	6.271	5.661	3.892	7.814	5.910
Often and deep	8.840	6.693	5.067	10.296	7.724

8. The Effect of Methods of Cultivation in 1933

In 1933 the Latin-Square test of 1931 was repeated, with the exception that only Rustler was used. It was planted in three-row plots with one-row borders between them. Each contained approximately 450 stalks. The seed was planted June 1 and 2, in soil in good physical condition. The seedlings emerged on June 7, and weather was favorable for their rapid development. Even tho weeds grew rapidly in the non-cultivated and listed plots, they did not appreciably check the growth of the corn plants until the last week in June when the corn was 15 to 20 inches tall.

The weeds were scraped from the non-cultivated plots on July 6, 7, and 8. The ridges of the listed plots were plowed down on July 8, and this so stimulated growth that the listed corn matured later in all replications than that of any other treatment.

At the end of the growing season the vigor of the corn in the cultivation plots was rated numerically as follows:

A. Often and deep	16
B. Check—usual cultivation	15
C. Listed—ridges plowed down July 8	12
D. None—weeds scraped out with hoes	7

The only noticeable difference in vigor between corn cultivated "often and deep" and "usual cultivation" checks was in the greater resistance of the former to wind.

The calculated severity of smut on these plots is presented in Latin-Square form in Table 17. It is evident from the data in this table that there was no apparent relation between the vigor of the corn and the severity of smut, and the analysis shows that the variance of error is greater than that due to treatments. Thus we can safely assume that the cultivation practices were not controlling factors in the end severity of smut on these plots.

Table 17.—Results of "Latin-Square" Cultivation Test in 1933

Cultivation and smut severity				Treatment means
A— 7.25	B— 6.93	C—5.78	D—6.84	A—7.85
C—10.17	A— 7.99	D—8.06	B—8.25	B—7.42
D— 8.22	C—10.16	B—8.31	A—9.70	C—8.35
B— 6.17	D— 8.90	A—6.44	C—7.39	D—8.01

9. The Seasonal Increase and Final Prevalence of Smut on Corn Planted on Different Dates in 1931

To study the influence of date of planting on the development of smut in 1931, four plantings were made at approximately 18-day intervals, namely, May 12, May 30, June 20, and July 6. There was only one row each of Rustler and Northwestern Dent in each series, and

there was only a single series in the June 20 planting because of lack of space. Counts of the number of smutted stalks were made on July 15 and are presented in Table 18, as a record of the seasonal increase in smut. The data also show the influence of date of planting on the prevalence of smut.

Altho the data in Table 18 are to a considerable extent self-explanatory, there are several things concerning them that perhaps merit comment. The reduction in prevalence of smut on the June 20 Rustler between July 25 and August 15 and on the May 12 Northwestern Dent between August 15 and September 15 requires explanation. The reduction on the June 20 Rustler may be explained by the disappearance of the early leaf smut because of withering and loss of the lower leaves in the hot, dry weather between the two counts. The reduction on the May 12 Northwestern Dent between August 15 and September 15 may also be readily explained by the early loss of tassels and leaves due to drouth. Northwestern Dent matures very early, even under normal conditions, and the tendency was accentuated in 1931 because of dry weather. It should certainly be justifiable therefore to use the August 15 figure, 43.4 per cent, rather than the 39.3 per cent, in comparison with other counts on September 15.

In order to appreciate fully the influence of date of planting on the prevalence of smut, one must make comparisons between plantings on the same age basis in so far as possible. For example, the August 15 percentage for the May 30 Rustler is more nearly comparable with the July 25 percentage for the May 12 Rustler than with the August 15 percentage for that planting, because the one planting is 18 days younger than the other. It must also be remembered, when one compares final percentages or severities, that late plantings do not have time to mature normally in Minnesota.

The data in Table 18 show clearly that the period of rapid increase of smut comes later for Rustler than for Northwestern Dent. It has

Table 18.—The Seasonal Increase and Prevalence of Corn Smut on Rustler and Northwestern Dent Varieties Planted on Four Different Dates in 1931

Variety	Date of planting	Date of count and prevalence of smut			
		July 15	July 25	August 15	September 15
Rustler	May 12	1.8 ± .299	2.1 ± .535	15.8 ± 1.073	40.6 ± 1.325
	May 30	3.5 ± .539	13.8 ± .701	16.3 ± .940	46.1 ± 1.059
	June 20	.....	3.6	2.6	33.4
	July 6	.....	.....	8.0 ± 0.069	13.4 ± 1.375
Northwestern Dent	May 12	2.3 ± .330	23.1 ± .971	43.4 ± 1.117	39.3 ± .755
	May 30	.6 ± .309	5.9 ± .765	35.3 ± 1.647	52.3 ± 3.249
	June 20	.....	3.5	35.9	52.4
	July 6	.....	.....	10.4 ± .587	36.3 ± 2.542

been the writer's observation that on Rustler the largest proportion of smut is located in the shoots or nodal buds and develops after the main stalk has ceased to grow. On Northwestern Dent the smut is well distributed but is perhaps predominantly on the tassels, necks, and ears, and, of course, develops when those tissues are meristematic. However, the principal importance of these data lies in their indication that late corn was more heavily smutted than that planted earlier.

10. The Severity of Smut on Corn Planted at Different Times in 1932 and 1933

In 1932 and 1933 the same plan as that for 1931 was carried out for the date-of-planting studies, but with the important exception that size of plot was doubled, making approximately 300 stalks per plot, and the plantings were on May 17, May 30, June 15, and June 30 in 1932 and May 17, June 6, and June 24 in 1933. The results, in terms of smut severity by the product method, are presented in Tables 19 and 20. The data given in Table 19 support the general statement of section 9 that the later the corn is planted the more heavily smutted it is likely to be, but they also present an important exception. There not only was not more smut on the May 30 planting than on that of May 17, but slightly less, altho the difference was not statistically significant.

It has been mentioned before that there was very little early-season smut on the May 30 corn in 1932, and it has been suggested that this is explainable by the tremendous rate of development of this planting during the first six weeks. The May 17 planting was retarded during the first two weeks by cool, dry weather and an unusual amount of leaf smut appeared on it.

Table 19.—The Severity of Smut on Rustler and Northwestern Dent Corn Planted on Four Different Dates in 1932

Variety	Date of planting and severity of smut			
	May 17	May 30	June 15	June 30
Rustler	5.250 ± .4099	4.937 ± .3197	10.291 ± .7930	8.487 ± .6085
Northwestern Dent	9.730 ± .5239	8.108 ± .2681	12.464 ± .5565	9.637 ± 1.0709

Table 20.—The Severity of Smut on Rustler and Northwestern Dent Corn Planted on Three Different Dates in 1933

Variety	Date of planting and severity of smut		
	May 17	June 6	June 24
Rustler	2.525 ± .3634	5.930 ± .1086	4.427 ± .3593
Northwestern Dent	4.525 ± .3290	13.275 ± .6598	6.180 ± .4626

In view of the negative results from the application of large amounts of inoculum, it may be fair to consider that a stronger tendency for spiral loosening and earlier development of lateral meristems are primary factors in the higher incidence of smut on late corn.

EXPERIMENTAL SECTION III

Studies on the Nature of Resistance of Corn to Smut

Needless to say, any information on the nature of resistance, particularly the relation of visible and measurable characters to smut resistance, might be of great value in corn breeding. For this reason, the two studies discussed below were made at University Farm in 1932.

1. Stage of Development or Unfolding at About One-foot Height

Brefeld (2, 3) and Hitchcock and Norton (13) early realized that the corn plant is more susceptible to smut at certain stages than at others. Brefeld concluded that plants about one foot tall were most susceptible, but Hitchcock and Norton stated that plants were more susceptible when about three feet high than at any other stage. It is probable, tho not definitely known, that Brefeld's conclusions were based on studies of sweet corn and those of Hitchcock and Norton on studies of field corn. If this is true, the discrepancy in the statements of Brefeld and of Hitchcock and Norton may be apparent rather than real, for corn varieties, especially of these two types, differ so much in size that one- and three-foot heights, respectively, may represent very nearly the same stage of growth.

The bulk of experimental and observational evidence collected by the writer indicates that sustained rapid development of the host favors resistance to corn smut under field conditions. That the tightly rolled, overlapping leaves of the rapidly unfolding plant maintain a covering and pressure which prevent inoculum from reaching susceptible tissues seems, at present, the best interpretation of this reaction (see Fig. 1), altho other factors may be very important. "Resistance" would then be considered disease escape rather than true resistance. If it is termed resistance, then it is a type due to gross morphology, which Miss Griffiths (9) and Platz (27) have held accountable for the resistance of corn to smut.

Miss Griffiths (9, p. 87) stated, ". . . . it is conceivable that resistance or susceptibility is largely a matter of relative accessibility of the susceptible parts to the invading organism."

Platz' interpretation (27, p. 197) of the relations between gross morphology, stage of host development, and smut reaction follows: ". . . . it seems evident that plants about one foot tall are more susceptible to infection by corn smut than either smaller or larger plants. The explanation, in all probability, lies in the difference in the morphology of the plants in these successive stages of growth. This difference in morphology may be noted by examining the transverse and longitudinal sections of plants in various stages of development. . . .

"In small corn seedlings the growing tip is very short and, together with numerous delicate leaf-primordia, is deeply set in the enveloping leaves that have unfolded. The unfolding leaves are rather firmly packed together in these plants. As the plants grow larger, however, the growing tip becomes longer, and the enveloping, unfolding leaves become less compact. At this stage of growth, the growing tip and the delicate leaf-primordia surrounding it are accessible, therefore, to a suspension of sporidia dropped into the characteristic terminal leaf-spirals formed by the unfolding leaves. It is the delicate leaf-primordia and the growing tip or tassel that become infected when plants are exposed by this method.

"On the other hand, plants two to four feet tall may, likewise, be morphologically protected against infection when a suspension of sporidia is dropped into their terminal leaf-spirals. Though the tassels of some of the plants at this stage of growth are still enveloped by the upper leaves they are almost completely developed by this time. It is possible, therefore, that their exposed surface is covered throughout with firm epidermal tissue, which the germinating sporidia may not be able to penetrate."

The smut reactions of a large number of selfed lines of different varieties and types of corn being known, it seemed to the writer that the determination of the height of the growing tip at the one-foot stage and the calculation of the coefficient of correlation between this measurement (or an index of this measurement in relation to the plant height) and the smut reaction should show how much the latter depends upon the accessibility of the growing-point at the one-foot stage, for the relative height at which the growing-point stands should perhaps be the principal factor in its accessibility.

Seed of a number of selfed lines that have been developed and tested in the cooperative corn breeding project of the Division of Agronomy and Plant Genetics and the Division of Plant Pathology and Botany was available.[5] In 1931 many lines were grown and measurements indicated the striking differences between lines with respect to the height of the growing-tip at the one-foot stage. However, most of the lines were so severely affected by drouth that the results could not have been considered representative, and the data were not analyzed. Eighty-eight lines were grown under favorable conditions in 1932, and in 81 of them there were 36 or more strong plants 20 to 40 centimeters tall when most of them were about 30 centimeters tall. The error introduced by the increasing rate of unfolding as the corn plant gets larger made it seem advisable to limit calculations to populations of plants ranging between 20 and 40 centimeters in height and to use the ratio of height of growing-

[5] The writer expresses his appreciation to Dr. H. K. Hayes and Dr. I. J. Johnson, Division of Agronomy and Plant Genetics, for seed of many of the lines.

point to height of plant to represent the accessibility of the growing-tip. Height of plant was taken as the distance between the first node and the level of the spiral receptacle capable of holding water. Height of growing-tip was taken as the distance between the first node and the growing-tip (leaves removed), or, if the tassel had developed considerably, distance from the first node to the base of the tassel. Figure 1 is a diagrammatic representation of the gross morphology of the corn plant at the one-foot stage and will aid in understanding the measurements taken. The growing-point index or ratio for each line was readily obtained by dividing the sum of growing-point heights by the sum of plant heights for each line population. The data are summarized in Table 21, which presents other data pertinent to the lines as well as the smut reaction. The latter is a four-year average for the majority of the lines and represents only prevalence, the percentage of plants smutted.

Table 21.—Smut Reaction, Growing-Point Index, and Joly Balance Reading of Selfed Lines of Different Origins

Line number (1930)	Variety or origin	Years selfed	Smut reaction		Growing-point index	Joly balance reading
			3- to 5-year average	July 22, 1932		
S-1	Minn. 13	8	10.2	0.00	.2660	1.389
5	do	7	52.1	.....	.2868	1.348
6	do	8	9.5	8.33	.2419	1.239
7	do	7	8.7	4.35	.2351	.....
18	Golden Bantam	7	27.4	0.00	.2539	.....
19	do	7	29.0	0.00	.2676	1.021
21	do	8	24.9	0.00	.1921	.....
22	do	7	22.3	0.00	.2044	.....
25	do	7	8.0	0.00	.2476	.....
32	Recombination	F_6	2.8	.....	.2697	.....
33	do	F_7	9.5	.....	.3648	1.323
34	do	F_7	23.2	10.91	.1725	.....
40	do	F_7	3.0	.....	.3360	.....
41	do	F_6	12.6	.....	.2645	.....
42	do	F_7	2.5	.....	.4322*	1.118
43	do	F_6	6.1	.....	.2672	.....
44	do	F_6	6.1	.....	.3927	.....
45	do	F_5	20.5	.....	.2764	.....
46	do	F_6	18.1	.....	.3082	.....
47	do	F_7	29.9	0.00	.2483	.....
50	do	F_6	41.7	.....	.3462	.....
53	do	F_6	5.8	.....	.1926	.....
55	Recombination	F_5	32.1	.....	.2747	.....
57	do	F_6	48.6	.....	.2478	.....
63	Jap. Hulless	7	24.7	.....	.1756	.....
66	do	7	6.9	.....	.2015	.....
69	do	7	14.4	.....	.2081	.....
70	do	7	19.6	.....	.2362	.....
90	Minn. 13	5	3.9	15.57	.2652	1.093
93	do	7	18.2	25.45	.2509	.....
95	do	7	30.4	12.60	.2962	.....
96	do	8	42.7	9.09	.3108	.....
97	do	8	3.4	17.92	.2124	1.220

Table 21 *(Continued)*.—Smut Reaction, Growing-Point Index, and Joly Balance Reading of Selfed Lines of Different Origins

Line number (1930)	Variety or origin	Years selfed	Smut reaction		Growing-point index	Joly balance reading
			3- to 5-year average	July 22, 1932		
98	do	8	14.8	31.94	.2533	0.927
104	do	8	39.0	2.59	.2429	1.220
107	do	8	20.4	0.00	.1715	1.297
110	Rustler	6	12.5	2.56	.2526	1.531
111	do	6	25.5	8.16	.2498*	0.922
112	do	5	8.9	0.95	.2501	0.991
116	do	8	36.5	13.76	.1954	1.211
117	do	12	47.8	33.67	.2333	1.146
118	do	10	52.4	32.11	.2193	0.997
120	do	10	15.8	4.35	.3052*	0.937
122	N. W. Dent	8	44.7	25.29	.2213	0.995
125	do	8	47.0	7.41	.1833	1.252
126	do	7	22.8	5.88	.2475	1.160
129	do	8	31.6	15.15	.2729	1.014
130	do	7	2.0	0.00	.2936	1.232
131	do	7	26.1	.....	.2760	1.052
132	Golden Glow	8	12.9	10.48	.2518	1.073
133	Silver King	10	24.2	1.25	.2924	.....
135	Robertson 13	8	65.9	6.82	.2475	1.362
138	Longfellow	11	12.2	0.91	.2896	1.498
139	King Philip	9	43.6	3.95	.2661	0.996
142	Golden Bantam	6	1.5	0.00	.2908	1.221
143	do	6	21.6	5.05	.4146	0.995
144	do	6	8.8	0.00	.3423	1.285
147	do	7	16.6	3.81	.2443	.....
148	do	8	25.7	5.26	.4136	.....
149	do	7	14.1	1.74	.3041	1.329
150	do	8	43.4	13.68	.3133	1.077
151	do	8	30.5	32.95	.3168	1.024
152	do	7	7.1	0.95	.2114	0.968
154	do	7	29.2	20.43	.2649	.....
155	Jap. Hulless	7	28.4	5.19	.2052	1.601
156	do	7	7.0	6.80	.1496	1.326
157	do	6	4.7	1.10	.1992	.....
161	Recombination	F_6	2.6	3.13	.2383*	1.116
162	do	F_7	19.7	12.84	.3375	0.990
164	do	F_6	13.4	16.88	.2845	.....
165	do	F_6	6.0	0.00	.3105	.....
166	do	F_7	10.5	3.13	.2731	.....
167	do	F_6	26.6	4.08	.3340	.....
169	do	F_7	21.4	5.79	.3596*	1.173
170	do	F_7	13.7	7.27	.2629	.....
172	do	F_6	10.3	1.54	.3569	.....
173	do	F_6	9.4	3.42	.2112	1.262
175	do	F_7	8.9	0.00	.3238	.....
176	do	F_7	40.8	4.39	.2457	1.001
177	do	F_7	20.8	3.05	.2655	1.056
178	do	F_6	17.6	6.14	.2355	.....
180	do	F_7	19.6	5.66	.2024	1.321
181	do	F_7	18.7	21.28	.2868*	.....
182	do	F_6	15.2	5.60	.2726	.....
183	do	F_7	15.1	3.75	.2493	1.069
185	do	F_6	23.3	6.06	.2293	.....
186	do	F_7	12.3	9.38	.2137	.....

* Less than 36 plants 20 to 40 cm. tall. Not used in calculating r between smut reaction and growing-point index.

Employing the formula, $r_{xy} = \frac{nS(xy) - S(x).S(y)}{\sqrt{nS(x^2) - [S(x)]^2}.\sqrt{nS(y^2) - [S(y)]^2}}$ for the calculation of the coefficient of correlation, it was found that r between average smut reaction and growing-point index was only $-.058 \pm .0747$. Altho not significant, this figure, being negative, suggests a stronger tendency for smut in those lines that developed slowly to the one-foot stage than in those that unrolled rapidly. Since it was evident that the selfed lines from field varieties unroll more slowly than those of the sweet-, flint-, and pop-corns, once the latter get started, r between smut reaction and growing-point index was calculated separately for these two classes of lines, on populations of 25 each. For the field-corn lines, r between smut reaction and growing-point index is $-.106 \pm .1334$, while for the sweet-, flint-, and pop-corn group it is $-.233 \pm .1277$.

It is interesting to note that r between growing-point index and the prevalence of early-season leaf, tassel, and stalk smut noted on the lines on July 21 and 22, 1932, is only $-.0886 \pm .0843$. These notes were available for only one season, but it was considered that the relation between the early-season smut and accessibility of growing-point might be much closer than that between total smut and the latter. Calculation of r was deemed worthwhile because 40 or more uniform plants, growing under very favorable conditions, were available in each of 63 lines, and it is believed that they constituted a representative sample.

From these results it seems clear that the height at which the growing tip stands at the one-foot stage plays very little part in the final smut reactions of selfed lines of corn. This may not be true for uniform hybrids, such as F_1 populations from homozygous parents. It also is possible that height of growing-point at a slightly later stage of development may be correlated with smut reaction. On the other hand, relative height of growing-point may be of no importance in the accessibility of smut-susceptible tissues to meteoric moisture, but this hardly seems possible.

2. Resistance to Puncture

Using a modified Joly balance, Hawkins and Harvey (11) found a correlation between resistance to puncture and resistance of potato tubers to certain fungous rots. Melander and Craigie (24), employing the same instrument, found that barberries very resistant to stem rust were also very resistant to puncture, tho there were exceptions. Since *Ustilago zeae* enters the corn plant by direct, apparently mechanical, penetration (39), it was suggested that resistance to puncture might, to some extent, determine the resistance of corn to this fungus.

During a cloudy week between May 8 and 15, 1932, preliminary studies with the modified Joly balance used by Melander and Craigie were made in the greenhouse on selfed-line plants that were very near the tasseling stage. In order that the results be more nearly comparable than they would be if too few measurements were made in different locations on a number of plants in different stages of development, plants were cut until one was found which had exposed the next to the last leaf. Since a comparison of the lines was the end desired, the direct reading in (centimeters) lowering of the support necessary to allow puncture of the epidermis was the figure used throughout. A steel needle, ground to 42 microns, the smallest diameter at which it did not break at right angles when rubbed over the finest stone available, was used in all the studies made with the balance. The results of these preliminary studies, presented in Table 22, indicated that there are marked differences between the lines but that a number of measurements for each line would be necessary because of the variation due to the differential response of the needle to the various structures of the leaf surface.

Table 22.—The Puncture Resistance of Leaves of Six Lines of Corn, of Known Smut Reactions, as Tested with a Modified Joly Balance, in Greenhouse, May, 1932

Line number (1930)	Average smut prevalence	Mean centimeters drop necessary to allow puncture
S-111	25.5	.6685 ± .0148*
S-142	1.5	.7702 ± .0155
S- 94	16.9	.8160 ± .0143
S-125	47.0	.9074 ± .0213
S-139	43.6	.9276 ± .0179
S- 1	10.2	1.2635 ± .0215

* With P.E. of this rank differences of .106 to .110 between means are 3 times their probable errors.

Long-time selfing notwithstanding, study of a carefully selected small portion of one leaf of one plant warrants no conclusions regarding a line, and the relationship between two characters of six lines, selected for other studies because of certain peculiarities, can not indicate a general relationship except by chance. The preliminary greenhouse measurements indicated that there are differences between the lines, and, on the basis of their indication, Joly balance readings were taken on 45 lines of the group being measured at the one-foot stage of development. The greatest care was exercised in selecting the material for testing. Four plants at about the one-foot stage were selected at random, and four readings were taken on each of four leaves from each plant, making a total of 64 measurements for each line. Leaves were peeled away until

young ones, having no more than one foot of blade exposed, were reached. The discarding was continued to the first leaf that was judged, from all previous observations on the development of smut, susceptible to infection and development of visible galls in blade tissue. On the first and largest leaf selected for measurement the sheath length rarely exceeded one inch, thus considerable elongation was yet to take place. The next three leaves inside were also sampled on each plant, four measurements per leaf being made on blade tissue between one-half and one inch from the ligule.

The data taken in this manner on the 45 selfed lines are presented by plant totals in Table 23 and by line means in Table 21. Analysis of the data in Table 23 by the variance method indicates that there are significant differences between the lines, the odds being infinite, and that differences of .137 or more between the line means presented in Table 21 may be accepted as significant. There is no correlation between smut reaction and resistance to puncture thus measured, however, for $r = -.095 \pm .0996$, which indicates that the observed differences in resistance to puncture have little to do with smut reaction.

Since it was concluded from histological studies (39) that the organism can penetrate tissues in which it apparently can not produce galls, the above negative results could, perhaps, have been anticipated. Moreover, the lack of correlation between smut reaction and resistance of leaves to puncture is in line with Brefeld's (2) early conclusion that the growth of the mycelium through the host is in any case limited, even tho it is only reasonable to suppose that the rate of extension will be slower in more mature tissues that are more resistant to puncture. Possibly the Joly balance readings measured rate of leaf maturity rather than the total possibilities for further meristematic activity in these lines of corn. The latest observations suggest that the latter is more important to gall formation.

Table 23.—The Joly Balance Readings,* by Plant Totals for 16 Measurements Each, on 45 Selfed Lines of Corn of Different Smut Reactions, Taken on Blade Tissue Within One Inch of the Ligules of Smut-Susceptible Leaves of Plants About One Foot Tall

Line number (1930)	Plant				Plant mean
	1	2	3	4	
S-1	20.66	21.27	21.96	25.00	22.22
5	23.26	23.55	18.63	20.86	21.58
6	19.41	20.06	18.86	19.99	19.88
19	17.41	15.84	16.15	15.97	16.34
33	17.18	20.67	26.01	20.83	21.17
42	19.29	17.73	16.17	18.38	17.89
90	15.96	15.94	19.17	18.90	17.49
97	18.68	19.84	18.22	20.31	19.51
98	15.66	15.84	14.17	13.67	14.84
104	18.62	19.40	20.43	19.64	19.52
107	22.78	22.12	19.28	18.81	20.75
110	31.47	19.96	23.26	23.28	24.49
111	14.47	14.99	14.93	14.60	14.75
112	14.33	15.14	15.81	18.16	15.86
116	20.09	19.34	19.58	18.49	19.38
117	18.61	17.05	19.41	18.27	18.34
118	15.88	16.73	14.70	16.37	15.95
120	15.10	14.35	15.56	14.96	14.99
122	15.95	15.95	15.62	16.15	15.92
125	19.10	18.36	21.77	20.88	20.03
126	18.94	18.38	18.90	18.00	18.81
129	16.59	16.41	14.68	17.24	16.23
130	19.55	18.54	21.03	19.53	19.71
131	15.62	15.39	17.38	18.93	16.83
132	16.91	16.79	17.33	17.62	17.16
135	20.03	19.48	22.57	25.10	21.80
138	26.43	33.29	24.75	21.37	23.96
139	16.13	15.86	15.83	15.93	15.94
142	21.65	19.93	18.74	17.81	19.53
143	17.10	15.59	16.69	14.32	15.93
144	21.15	19.41	21.51	20.18	20.56
149	23.21	21.56	20.22	20.04	21.26
150	17.88	16.97	16.22	17.88	17.24
151	16.05	16.56	15.75	17.15	16.38
152	15.90	14.53	15.26	16.25	15.49
155	25.51	28.44	25.08	23.42	25.61
156	20.51	20.47	22.82	21.07	21.22
161	18.36	18.02	17.25	17.81	17.86
162	16.01	16.52	15.10	15.75	15.85
169	17.86	18.62	18.51	20.09	18.77
173	21.44	19.48	20.83	19.04	20.20
176	15.44	17.00	16.41	15.22	16.02
177	16.28	17.46	17.98	15.85	16.89
180	23.04	20.03	21.34	20.14	21.14
183	19.21	16.82	15.31	17.08	17.11

* A steel needle ground to approximately 42 microns in diameter was used for all the tests.

DISCUSSION AND CONCLUSIONS

Résumé of Experimental Results

An assembly of the observations and conclusions on questions treated separately in the preceding pages is now desirable to pave the way for discussion, broader conclusions, and interpretations. In recapitulation, the following outstanding results and deductions from the experiments concerning factors influencing smut development may be listed:

Topping or detasseling greatly increased the prevalence and severity of smut on certain varieties or lines if done when considerable growth was yet to take place. Many selfed lines and F_1 populations obtained by selfed-line mating did not respond to this treatment; therefore, the reaction is very likely dependent upon host genotype. Mutilation by slashing markedly increased smut severity when practiced on corn at an intermediate stage of rapid development but was ineffective on less mature and on more mature plants. The internodal meristems and lateral shoots rather than the injured surfaces were involved in smut increases due to mutilation (both topping and slashing).

The application of inoculum by spraying, dropping, pouring, or dusting, involving the transfer of either or both sporidia and chlamydospores to the portions of the plant reached by meteoric water, did not increase the prevalence and severity of smut above natural infection. This was true of plants injured by slashing as well as non-injured plants, i.e. injury plus inoculum did not increase smut above injury alone.

The effects of injuring by rolling leaf-spirals between the palms of the hands, a simulation of the spiral-loosening effect of twisting in the wind, were more striking on the later than on the early plantings. Increases were evident on the latter but were primarily in leaf and tassel galls, which are commonly dropped by early planted corn before the end of the growing season.

Sterilized water or broth injected into plants between the 10- and 20-inch stages increased the severity of smut greatly, apparently by making connection between the inoculum-containing meteoric water of the upper leaf-spiral and the susceptible tissues lower in the roll of leaves, for puncturing alone does not increase smut and injection of dry chlamydospores results in severe smut. The smut-producing effectiveness of the injection of sterilized liquids increased with the advance of the season. However, injection of sterilized liquids, producing striking increases in the total amount of smut, did not, on Rustler, increase the severity of nodal-bud smut, which is by far the most common type of smut in general. Thus it seems fair to consider the problem of smut reaction quite largely a question of the gall-producing effectiveness of

inoculum at University Farm. This leads to the further consideration that the influence of environment on the plant is of very great importance to its smut response.

Plants which had early-season leaf smut, of little consequence in itself, produced more late-season nodal-bud smut than the average plant, the data applying to the Rustler variety, on which nodal-bud smut is common.

Infection directly through young husks evidently accounted for ear smut in certain lines but is apparently not very common.

The effects of injuring by slashing were much more definite in 1932 and 1933, when the plants were injured during rain, than in 1931 when corn was injured during the evenings or early mornings of dry days. It seems most likely, however, that this difference was due to the indirect effect, the host growth response following rain, rather than to the effect on the pathogen, for smut was more prevalent in 1931 than in either 1932 or 1933, and application of inoculum to injured plants failed to increase smut severity above injury alone in all three seasons.

The most rapid increase of smut on Rustler, a variety adapted to southern Minnesota, comes later in the season than the period of most rapid increase on the Crookston strain of Northwestern Dent, an early-maturing variety adapted to northern Minnesota. Galls are well distributed on Northwestern Dent, but on Rustler are principally of the nodal-bud type.

In general, corn smut was more prevalent and more severe on later plantings than on early plantings. In 1932 smut was slightly less severe on corn planted May 30 than on the May 17 planting, but the former grew unusually rapidly, being under very favorable conditions during the first six weeks.

Manure and commercial fertilizers, separately and in various combinations, have failed thus far to affect smut development very significantly; but growth responses to the soil treatments have been lacking or negligible. In 1931, a very dry season, nitrogen reduced the amount of smut while manure+nitrogen+phosphorus+potassium and nitrogen+phosphorus increased it on Northwestern Dent. The former caused the plants to grow more rapidly during early summer, while the latter two treatments caused earlier drying. In 1933, manure distinctly affected the vigor of Rustler, but there was no significant alteration of smut severity.

Corn of the Rustler variety on a fertile field developed less smut than the same strain planted near the same date on a less fertile field in 1932.

Cultivation practices markedly affected the vigor of corn in 1931, and on Rustler, especially, the prevalence of smut was not proportional to

the vigor of the corn. With the exception of very severely stunted, listed Rustler, prevalence of nodal-bud smut was inversely related to vigor on both Rustler and Northwestern Dent.

There is no correlation between the relative height of the growing-tip in plants about one foot tall and the smut reaction of selfed lines tested and measured at University Farm.

No correlation could be detected between resistance of leaves to puncture and smut reactions of selfed lines.

Consideration and Discussion

As was stated in the introduction, elucidation of the nature of resistance of corn to smut was the primary objective of the studies that have been described in the preceding pages. Some of the experiments were, however, of a type that yielded results of practical application in the cultivation of corn, notably the effects of injury by topping or slashing plants in the intermediate stages of rapid development. Similar results on this point have been obtained earlier by Clinton (5) and Piemeisel (26). The results of all the field-plot tests could, seemingly, best be summarized for application to cultural practice by stating that those conditions and treatments which favored the sustained rapid growth of the plants from emergence to maturity regularly resulted in less smut than conditions and treatments that were unfavorable to host development.

In the writer's experience, corn which grew rapidly and consistently developed less smut than that less favored by soil and weather conditions. This is not in agreement with the general conclusions in other available reports, except that by Mazé and Mazé (23); and the details on which the latter base their conclusion are not known. On the other hand, the safest generalization, perhaps, which one can make concerning the development of corn smut is that the more succulent the plant the larger the smut gall. The lack of agreement between workers may be partly due to the failure of some to consider the different effects of galls of different sizes.

It is not safe to conclude that vigor is directly associated with smut susceptibility, for the preponderance of the writer's evidence is to the contrary. However, it must be admitted that Kyle's warning to corn breeders that smut resistance in some cases may be due to a lack of vigor has some support in the writer's observations. His conclusion may be applicable to a lower reaction level if we consider that hybrid or normal variety corn represents the higher level of vigor, and that selfed lines, with which may be classed severely stunted normal variety corn such as

the listed Rustler of 1931 (see Table 14), represent a lower vigor reaction level. The most resistant line with which the writer has dealt (S-142 listed in Table 21) has been consistently weaker and slower in growth than closely related, more susceptible lines; there are undoubtedly similar examples within the group studied.

The writer's observations on the development of corn smut have led him to the opinion that the best interpretation of the foregoing results and conclusions hinges upon recognition of the fact that the smut gall is the product of both host and parasite. The fungus produces smut galls by stimulating host cells to abnormal division and enlargement, and cells beyond a certain stage of maturity are evidently not susceptible to such stimulation. Can this not account for the thin or wart-like galls produced by certain lines, just as it accounts for the small, thin galls on the more mature tissues of susceptible lines? May it not be that weak but highly resistant lines lack the reserve necessary as an impetus to the production of the laterals requisite for large galls?

Why did the plants which were heavily dusted with chlamydospores so consistently develop less smut than the checks, exposed to natural inoculation only? Possibly heavy inoculation induces a necrosis of host tissues which, in the manner of a hypersensitive reaction, protects the plant from further advance by the smut hyphae, as the writer (39) has previously suggested on the basis of histological studies. Repeated observations likewise suggest the possibility that a balance between necrotic and stimulatory effects of smut hyphae is operative in the relatively inactive lateral meristems of rapidly developing plants. This entire problem of the effects of heavy inoculation and necrosis of host tissues needs further study and careful scrutiny before judgment is passed.

If it is kept in mind that the fungus can cause production of conspicuous smut galls only in meristematic tissues, can not all of the foregoing results and observations be combined into one system of simplified significance? In view of the failure of large amounts of inoculum to increase smut severity, may not the effect of mutilation be attributable to its influence on the development of lateral and internodal meristems? Is not the greater development of nodal-bud smut on Rustler that has been retarded in growth and ripened prematurely on sloping land than on Rustler growing consistently in moist soils most likely due to increased lateral meristem activity? Since we do not yet know any lines with convincing physiological resistance or immunity, Ranker's report (29) notwithstanding, it would seem that differential cell division or meristem activity of the lines and varieties studied might explain differences in their reaction to smut. Kyle's (21) observation that all the potential-ear smut on his furrow-planted corn was at the second and

third nodes, the points between which the plants were most retarded in growth, may, it seems, be best explained by the greater tendency for lateral-shoot development in this stunted portion of the stalk.

The foregoing indications, observations, and conclusions that reaction to smut, or smut escape, is frequently controlled by growth responses, the interactions of host and environment, are by no means final. But they show clearly that there are two phases of growth of the corn plant which are very likely important to smut reaction. It has been observed that *(a)* sustained rapid development of plants to full stalk size favors resistance or escape and that *(b)* development of smut in later season depends on lateral meristem activity. There also is evidence from the responses of the varieties and lines used that one of the above responses may be far more important than the other in determining the reaction of a particular line or variety. Perhaps the most striking thing concerning the reactions of carefully studied lines and varieties is the fact that location of galls is characteristic for many lines, and it is very well known that the characteristic location of galls, as well as the total amount of smut for a given line or variety, may vary considerably with the season, region, or date of planting (14, 15). Considering the development of the corn plant in this connection, it is immediately realized that a time factor may be very important in the smut response of many lines and varieties of corn.

In conclusion it may be stated that the interpretation that has been presented for the interacting host-environment influence on smut development finds precedence and support in well known and accepted facts concerning the development of smuts of small grains. Three fairly well known growth-response influences on smut development may be mentioned: *(a)* The common observation that the strongest tillers of bunted wheat plants are often free of smut; *(b)* the effect of fertilizer treatments favoring host vigor in reducing the prevalence of smuts of oats; and *(c)* Brefeld's (3) early observation that rapidly developing seedlings of oats and sorghum produced fewer smutted plants than did comparable series in which growth was slower immediately following germination. The results presented in this bulletin show clearly that the stage of development of the plant at which it is mutilated, inoculated, or at which its growth is arrested by unfavorable conditions is of the utmost importance to the smut response. These results increase the importance of the host-environment interaction and decrease the significance of the pathogen in the development of corn smut; however, future progress on the problem of nature of resistance will, perhaps, only come by a better understanding of the relations between this variable organism and its heterozygous host.

SUMMARY

1. Mutilation by slashing and by detasseling (or topping) markedly increased smut severity when practiced on corn at an intermediate stage of rapid development but was ineffective on more mature and on less mature plants. The inter-nodal and lateral meristems, rather than the injured surfaces, were involved in the increases, and response to the detasseling or topping is evidently dependent upon host genotype.

2. The application of large amounts of inoculum to portions of the plant reached by meteoric water has not increased the prevalence and severity of smut above natural infection at University Farm, St. Paul, Minnesota. This has been true of injured as well as non-injured plants during four seasons, 1930, 1931, 1932, and 1933. It is concluded that there usually is an abundance of inoculum and that the degree of its effectiveness is markedly affected by environmental factors influencing the host as well as by inherent characters of the host.

3. Injection of sterilized water into the leaf spirals of plants between the 12- and 20-inch stages greatly increases the total severity of smut, especially in the latter half of the season, apparently by making connection between the inoculum-containing meteoric water of the upper leaf-spiral and the susceptible tissues lower in the roll of leaves. This treatment did not increase the severity of nodal-bud smut on Rustler above natural infection, however.

4. Rolling the leaf-spirals between the palms of the hands during or immediately following rain, a simulation of the spiral-loosening effect of twisting in the wind, was effective in increasing the severity of smut if practiced on plants between the 12-inch and early boot stages. The effectiveness of this method of injury increased with the advance of the season, as was true for the injection of sterilized liquids, and the *modus operandi* is interpreted to be the same as for the latter method.

5. Plants of the Rustler variety having early-season leaf smut, of little consequence in itself, developed more late-season nodal-bud smut than plants that escaped early-season smut.

6. Direct infection through young husks causes ear smut in some lines but apparently is not very common.

7. Plants of Rustler variety, growing vigorously on low land well supplied with moisture, developed less smut than plants treated similarly but growing on land lacking moisture necessary for sustained growth. However, Northwestern Dent under the same conditions did not respond in the same manner.

8. The most rapid increase of smut on Rustler, a variety adapted to southern Minnesota and most commonly smutted in the nodal buds,

comes later in the season than the period of most rapid increase on the Crookston strain of Northwestern Dent, an early-maturing variety adapted to northern Minnesota. Galls are ordinarily well distributed on Northwestern Dent.

9. In general, smut was more destructive to later plantings than to early plantings, altho there was an exception on certain plantings in 1932.

10. Commercial fertilizers and manure did not cause pronounced growth responses of corn during the four seasons of these experiments, and smut development was not significantly affected by them. However, Rustler, growing vigorously on a fertile field in 1932, developed less smut than the same strain planted about the same time on a less fertile field.

11. Cultivation practices decidedly affected the vigor of corn in 1931, but the total prevalence of smut was not related to the vigor of the plants. With the exception of very severely stunted Rustler, growing in listed plots, the prevalence of nodal-bud smut was inversely related to vigor in both Rustler and Northwestern Dent.

12. A direct relationship between the vigor of host and susceptibility to smut has been noted only for plants of extremely low vigor, such as the above-mentioned Rustler growing in listed plots and certain very weak selfed lines.

13. There is no correlation between the relative heights of the growing tips at the one-foot stage and the smut reactions of 81 Minnesota selfed lines representing field-, sweet-, flint-, and pop-corn varieties.

14. There is no correlation between the resistance of their leaves to puncture, as measured by a Joly balance with the needle 42 microns in diameter, and the smut reactions of selfed lines which are distinctly different in both respects.

15. With regard to the influence of the interaction of host and environment, it appears that sustained rapid development of plants to full stalk size in many cases favors escape from, or resistance to, smut, and that development of large smut galls later in the season depends on lateral meristem activity.

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